Ecological stoichiometry of consumer-resource interactions in lotic food webs

by Jennifer Melinda Moslemi

ECOLOGICAL STOICHIOMETRY OF CONSUMER-RESOURCE INTERACTIONS IN LOTIC FOOD WEBS

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The natural world is inherently complex, and the application of theories that reduce complexity may reveal general biological patterns that are otherwise obscured. The theoretical framework of ecological stoichiometry provides a lens through which to broaden understanding of complex ecological dynamics by distilling them into mass balance relationships. Using ecological stoichiometry theory, ecological interactions can be understood as the quantifiable exchange of energy and multiple chemical elements that are transformed but not destroyed. Here, I use ecological stoichiometry to understand how: (1) excreted nutrients by an invasive herbivore influence nitrogen dynamics in tropical streams, (2) physical, chemical, and biological factors define the elemental composition of food webs across regional scales, and (3) the elemental composition of lotic food webs responds to manipulations of top-down and bottom-up forces at different experimental scales.

In Trinidad, West Indies, I quantified the influence of an invasive snail (*Tarebia granifera*) on nitrogen cycling in tropical streams. I found that snail biomass, growth rates, and excretion rates were greater in locations where riparian canopy was open, most likely due to an increased quantity of food resources in open canopy sites. Measurements of ecosystem demand for nitrogen showed that snail excretion supplied a larger proportion of ecosystem demand in locations where riparian canopy was open, suggesting that restoration of riparian canopy may

ameliorate the effects of invasive snails on nitrogen cycling.

In the Colorado Rocky Mountains, I measured several physical, chemical, and biological attributes of streams across a watershed to determine which factors predicted the carbon, nitrogen, and phosphorus stoichiometry of consumers and their resources in sub-alpine streams. I found that physical factors that characterized size, temperature, and disturbance regimes best described the stoichiometry variability of food webs.

In Colorado I also tested the effects of nutrient availability, grazing pressure, and predator cues on the carbon, nitrogen, and phosphorus stoichiometry of consumers and resources across experimental scales. Added nutrients (nitrogen and phosphorus) consistently increased phosphorus content of epilithon, and grazers consistently increased epilithon carbon and nitrogen content. Predator cues reduced the influence of grazers on epilithon stoichiometry only in small-scale experiments.

BIOGRAPHICAL SKETCH

Jennifer Melinda Moslemi was born in the town of Moscow in the Idaho panhandle. As a young girl traveling with her parents to visit family scattered around the world, Jennifer developed a love for discovering new landscapes and cultures and decided that her future career must not deny her budding wanderlust. After high school, Jennifer knew it was time to leave her beloved hometown and seek the new. She attended the University of Washington in Seattle for her undergraduate studies, where she found herself both bewildered and empowered by her new school of 40,000+ students. As soon as she flew the nest, Jennifer's parents seized the opportunity to move temporarily to Costa Rica, where Jennifer's dad worked at a postgraduate institution. It was on visits to Costa Rica to see her parents where Jennifer first became enthralled by living organisms and their ecosystems. She pursued biological studies and was fortunate enough to find herself in a course taught by Drs. P. Dee Boersma and Daniel E. Schindler in which students pursued independent research projects. With the guidance of Drs. Boersma and Schindler, Jennifer became fascinated with science and its application to the natural world. Upon graduating, Jennifer scored an opportunity to work with Dr. Boersma on Magellanic penguins in Patagonia. There she earned her field biology stripes while gathering data on the beautiful but often ill-tempered birds. Once back in the States, Jennifer took a job with the National Oceanic and Atmospheric Association working on risk assessment of threatened salmon populations in the Pacific Northwest. Jennifer's inspiring mentors at NOAA helped solidify her desire to pursue postgraduate studies. Knowing that she wanted to combine her love of travel, field biology, and freshwater science, Jennifer found her way into the lab of Dr. Alex Flecker at Cornell University who encouraged and challenged her to develop her scientific skills and pursue her

interests across the globe. While at Cornell University, Jennifer tragically lost her beloved Jarrett and took an indefinite break from her studies. Jennifer's family, friends, and mentors would provide her with invaluable and unconditional support during this time, and Jennifer later decided to return to Cornell to finish her PhD. There Jennifer was inspired to be a better human being and scientist by her many admirable friends and mentors, and conducted research in Trinidad and Tobago, Bolivia, Venezuela, and the Colorado Rocky Mountains.

For my parents, whose soulful support, love, and encouragement have made me feel like I won a cosmic lottery

And

For Jarrett and the inspiration that he continues to be

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Dissertations are the product of a community effort, and mine is certainly no exception. There are many people without whose guidance, persistence, and encouragement this work would not have been possible.

My heartfelt thanks to my advisor, Alex Flecker, whose counsel, encouragement, and support has been an invaluable constant during my time at Cornell. His integrity and enthusiasm have been infectious, and I feel lucky to have been near enough to hopefully catch some of it. I also am deeply grateful to my committee members, Bobbi Peckarsky, Nelson Hairston, and Daniel E. Schindler who have been generous with their insight. I have worked closely with Bobbi for a few years in the awe-inspiring Rocky Mountains, and have learned lessons about hard work, steadfast leadership, and of course, wholehearted play that I will carry with me. Nelson many times provided a much-needed light in the dark when I became lost in my ideas. Daniel was a mentor during my undergraduate years, and I treasured his guidance so much that I asked him to stick with me through graduate school. I am also immensely grateful for the friendship of Harry Greene, who has been generous with his wisdom and contagious with his optimism.

The Flecker lab has been at the forefront of my academic family, and has provided consistent quality control, helpful ideas, and constructive criticism. I have benefited greatly from interactions with Pete McIntyre, Steve Thomas, Rana El-Sabaawi, Bryon Daly, Amber Ulseth, Krista Capps, Marita Davison, Mike Booth, Sarah Collins, and Chris Dalton. I am also grateful to my extended academic family in the Hairston lab for intellectual and logistical support, and in particular thank Colleen Kearns, Joe Simonis, Cayelan Carey, and Becky Doyle-Morin.

I chose to embark on an ambitious series of field projects, which would have

been impossible with the hard work of many people who were generous with their time and energy. In Trinidad I was given a helping hand by Sunny Snider, Jim Gilliam, Rana El-Sabaawi, Marita Davison, Sarah Collins, and Tom Heatherly. Dena Vallano kindly measured thousands of invasive snails on her tropical vacation. In Colorado I was assisted by Wendy Brown, Marge Penton, Maruxa Alvarez, Marita Davison, Matt Fuller, who I thank not only for their effort but friendship and enthusiasm. Thanks also for the logistical help of the staff at the William Beebe Tropical Research Station in Trinidad and the Rocky Mountain Biological Laboratory in Colorado. A special thanks to billy bar at RMBL for being billy bar.

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Finally, my utmost thanks to my friends and family, whose soul-lifting support and encouragement has been an instrumental catalyst for everything that I have done. Thanks to my friends at Cornell who were always at the ready with a bottle or two of good wine and a caring ear when the situation called for it. They have and continue to inspire me to be a better human. A special thanks to Marita, who challenged me to live wholly again, and has brought bright colors to my life. My parents Al and Sousan Moslemi have been unwavering in their wisdom, support, and unconditional love. They nurtured my curiosity as a young child and provided a loving springboard from which I could chase my passions. I continue to be amazed the abundance of spirit and compassion of my mom, who inspires me daily.

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CHAPTER 1

IMPACTS OF AN INVASIVE SPECIES ON NUTRIENT CYCLING IN TROPICAL STREAMS: THE UNANTICIPATED ROLE OF RIPARIAN DEFORESTATION

ABSTRACT

Non-native species and habitat degradation are two major catalysts of environmental change and often occur simultaneously. In freshwater systems, degradation of adjacent terrestrial vegetation may facilitate invasion by altering resource availability. Here we examine impacts of an invasive herbivorous snail, *T. granifera*, on nitrogen (N) cycling in tropical stream systems. We also investigate the potential for riparian canopy removal to create hotspots of snail N excretion by altering food resource quantity and quality. In a set of Trinidadian streams, we measured snail biomass and N excretion in open and closed canopy habitats to generate estimates of mass- and area-specific N excretion by invaders. Snail biomass was 2 to 8 times greater and areal N excretion ranged from 3 to 9 times greater in open canopy relative to closed canopy habitats. Snails foraging in open canopy habitat also had access to more abundant food resources and exhibited greater growth and mass-specific N excretion rates. Estimates of ecosystem NH_4 demand indicated that snail N excretion supplied 2%, 11%, and 16% of demand in closed, intermediate, and open canopy habitats, respectively. We conclude that under some ecological conditions riparian canopy loss can generate hotspots of snail biomass, growth, and N excretion along tropical stream networks, thereby exacerbating invasive snail impact on an important biogeochemical cycle.

INTRODUCTION

Species introductions and habitat degradation are two major components of environmental change (Vitousek et al. 1997, Mack et al. 2000). Numerous studies have documented the impacts of invasive animals at various levels of biological

organization (Moulton and Pimm 1983, Vitousek 1990, Baxter et al. 2004); however, consequences for nutrient fluxes have been relatively understudied despite significant implications for fundamental ecosystem processes. Invasive animals may directly alter nutrient fluxes through consumption and excretion (Arnott and Vanni 1996, Vanni 2002) or indirectly via predation (Schindler et al. 1997). In aquatic systems there are relatively few examples that directly link effects of exotic animals on nutrient fluxes to ecosystem-scale processes (e.g. Strayer et al. 1999, Hall et al. 2003). However, both local extinction of native species and addition of exotic species may impact nutrient fluxes by altering biomass (Hall et al. 2003) or assemblages of species-specific traits such as nutrient excretion ratios (Arnott and Vanni 1996, McIntyre et al. 2007). Notably, studies of biogeochemical effects of animal invaders in tropical regions are especially sparse (but see Carlsson et al. 2004). This is despite evidence that nutrient cycles and controls on primary production may differ between tropical and temperate systems (Downing et al. 1999), indicating that results of studies conducted in temperate zones cannot be simply extrapolated to tropical cases.

Contemporary species invasions occur in the context of rapidly changing landscapes. Habitat destruction caused by land conversion is a major agent of environmental change that occurs simultaneously with invasive animal dispersal and establishment, often with facilitating or unknown effects on invaders (Meyerson and Mooney 2007). Yet consequences of habitat loss and animal invasions are most often studied independently of one another, without consideration of potential interactions (Didham et al. 2007). Lack of understanding of interactive effects hinders efforts to predict and manage invasions at multiple scales. Identification of land use changes that unintentionally facilitate invaders yet can be reasonably curtailed or reversed is crucial to formulating mitigation strategies that increase often-daunting odds of success. For aquatic systems, degradation of riparian forests adjacent to water bodies

is of heightened importance because of the potential for large alterations to resource flux between terrestrial and aquatic ecosystems (Likens and Bormann 1974, Nakano et al. 1999). Riparian systems have been extensively degraded by human activities for centuries (Naiman and Decamps 1997). Disturbances that decouple nutrient exchange between terrestrial and aquatic ecosystems can mirror impacts of exotic species on food webs (Baxter et al. 2004), although it remains unclear whether the effects of aquatic invaders in the context of riparian degradation can have interactive properties that exceed the additive effects of threats.

Here we studied the influences of an introduced herbivore/detritivore, the quilted melania snail (*Tarebia granifera*, see Illustration 1.1), on nitrogen (N) cycling in tropical streams. N is an important and potentially limiting or co-limiting nutrient for primary production in tropical streams (Flecker et al. 2002, Elser et al. 2007), and effects on N cycles can therefore have marked consequences for aquatic food webs and the services they provide for tropical regions. We measured N excretion by invasive snails and ecosystem N demand to estimate the degree to which *T. granifera* influences N cycling. We compared snail excretion across habitats along stream reaches where riparian vegetation was either present or removed to determine whether excretion rates were associated with loss of riparian vegetation. Lack of vegetation could be hypothesized to have opposing impacts on herbivore/detritivores: it may augment sunlight availability and lead to increases in algal biomass, but also inhibit flux of food resources from terrestrial ecosystems. These analyses contribute important information about the effects of riparian degradation on spatial patterns of nutrient cycling by invasive animals.

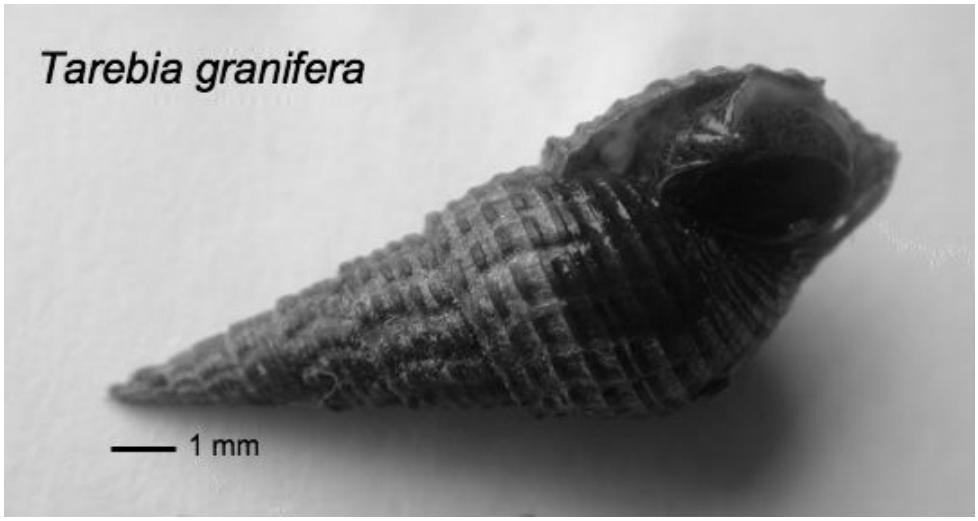

Illustration 1.1. The quilted melania snail (*Tarebia granifera*) is a proliferate invasive species that has established in freshwater habitats throughout much of the Neotropics. Photo credit: S. B. Snider.

METHODS

Study species

Introduction of *T. granifera* (Gastropoda: Thiaridae) to the West Indies occurred via both accidental and intentional pathways. Initial introduction of this parthogenetic snail to the Neotropics from Asia is presumed to have been an unintentional consequence of the aquarium trade, though intentional introductions have occurred in the West Indies in an attempt to reduce schistosomaisis outbreaks (Pointier and McCullough 1989). Although *T. granifera* does not act as an intermediate host to schistosomes, it may for other trematodes that detrimentally impact the health of aquatic animals and humans (Abbott 1952). *T. granifera* is now found worldwide throughout the topics and subtropics, though the ecological effects of invasion remain unknown.

Study site

We focused efforts on three streams for this study—Ramdeen Stream, Aripo River, and Yarra River—from separate drainage basins in the Northern Range mountains on the island of Trinidad, West Indies (10°41' N, 61°17' W; streams hereafter referred to as RAM, ARI, and YAR). RAM and ARI are located on the southern slope of the Northern Range, and YAR is on the northern slope. All three streams are fast-flowing and shallow with little aquatic macrophyte vegetation. Nutrient diffusing substrates (*sensu* Tank et al. 2006) placed in RAM in 2007 indicated N and phosphorus co-limitation of primary production (Appendix 1A). The substrate is dominated by cobble and gravel, and canopy cover is variable. Surrounding land use includes secondary forest, agriculture, and residential development.

Percent canopy cover over streams was determined using a densiometer (Type A convex, CSP Outdoors, Shreveport, LA, USA). "Open canopy" habitat was designated as 25% canopy cover or less, "closed canopy" habitat was 75% canopy cover or greater, and remaining values (26 – 74% canopy cover) were categorized as "intermediate canopy". The quantity and quality of food resources for invasive snails were characterized in each of these habitats by measuring the biomass of organic matter (chlorophyll *a* and ash-free dry mass) and carbon (C) and N composition of epilithon, respectively (Appendix 1B). *T. granifera* densities were estimated for each habitat type from randomly assigned transects (RAM) or quadrats (ARI and YAR) using a hand net (2 mm mesh size; 5 total sites for each habitat type). Snails smaller than 2 mm were not included in the study. Cross-stream transects of a constant width (0.15 m) from the edge to the midpoint of stream wetted width were used in RAM (n = 16). Quadrats (0.33 m^2) in ARI (n = 16) and YAR (n = 12) were divided evenly between random locations across the width of streams to account for edge effects. Total length of individual snails was measured to the nearest 0.1 mm and biomass measured as mg of ash-free dry mass (AFDM) was calculated using a length-mass regression (log AFDM = 2.35L – 1.83, where L is log of shell length in mm; n = 34, r^2 = 0.98) or an aperture-mass regression if shell tips were eroded or broken (log AFDM = 2.45A – 0.08, where A is log of shell aperture width in mm; n = 25, r^2 = 0.96). The regression accounts for organic matter within the snail, but not the inorganic shell of the parent snail or of any unborn offspring.

We measured N excretion rates of *T. granifera* using methods modified of Hall et al. (2003). In 2008 individual snails were placed into 20 ml clear plastic vials filled with filtered stream water. Snails were collected during daylight hours from open and closed canopy sites and immediately incubated in vials for 1 h in the field. After the incubation, water samples were filtered (Pall-Gelman A/E) and analyzed for NH_4

concentration using fluorometric methods described by Taylor et al. (2007). We focused on NH_4 because it is the dominant N compound excreted by aquatic snails and is readily available for uptake by primary producers. Snails represented the size distribution of *T. granifera* found in each stream (RAM: $n = 124$, ARI: $n = 40$, YAR: $n = 40$). We controlled for background NH_4 concentration in stream water and any unanticipated vial effects by subtracting the background NH_4 concentration in vials incubated without snails (RAM: $n = 16$, ARI: $n = 4$, YAR: $n = 4$). Some fraction of calculated NH_4-N excretion rates may have been due to leaching from egested fecal matter. Egestion was not quantified but fecal production was low over the course of incubations. We estimated areal N excretion rates by multiplying mass-specific excretion rates by average snail density for each snail size class and then aggregating across all size classes.

To determine if *T. granifera* growth rates differed between canopy types, we measured growth rates of snails in open and closed canopy sites using a reciprocal transplant design in RAM. We chose an open canopy and closed canopy site separated by at least 30 m. We collected 50 snails from the open canopy site; of those, half were placed in the same site (as a control) and the remaining half were transplanted to the closed canopy (reciprocal) site. The same procedure was repeated for a separate sample of fifty snails in the closed canopy site. Snails were collected over a broad area within each canopy site to ensure that the sample was representative of the source population. Snails were placed in groups of five into separate flow-through containers (Toby Tea Boy, Plymouth Tea Co., West Chatham, MA, USA) along with substrate collected from the incubation site to alleviate potential starvation effects on growth rates. Length of each snail was measured before the experiment and after 10 days of incubation. Growth rates were calculated as $(\ln M_t - \ln M_0)/t$ where M_t was the mass of an individual snail after incubation and M_0 was the mass of the

same snail before incubation (Hall et al. 2006). We used response ratios to estimate effect sizes and 95% confidence intervals of biomass-specific snail growth rates (Hedges et al. 1999): $\ln(X_t/X_c)$, where X_t is the mean growth rate for transplanted snails and X_c is the mean growth rate for control snails.

To put *T. granifera* NH_4-N excretion into the context of ecosystem-level demand for NH_4-N, we measured area-specific NH_4-N uptake rate in RAM in February 2008 and 2010 (Newbold 1981, Appendix 1C). NH_4-N demand was measured using a short-term solute addition (Tank et al. 2006) in which known concentrations of NH_4-N (as NH_4Cl) and a conservative tracer (NaCl) were simultaneously released at a constant rate via a peristaltic pump (Fluid Metering, Inc., Syosset, NY, USA). The decline in concentration of NH_4-N was measured after correction for background concentration and non-biotic sinks (using the decline in conservative tracer as an estimate). Solutes were released until conductivity reached plateau at the downstream end of the study reach (~1 h). Eight to 10 sampling stations were set up every 10 m along a 100 m study reach, and NH_4-N samples and conductivity measurements were taken at all stations before solutes were added (to establish background concentrations) and once conductivity had reached plateau (n = 3 per station). Conductivity was measured using a YSI 85 meter (Yellow Springs, OH, USA). Water samples were placed on ice and analyzed fluorometrically within 4 hours of sampling. Mass of NH_4-N added to the stream was calibrated to be detectable using fluorometric techniques but not in sufficient quantities to alter pathways of uptake over the time period of the addition (~2 h). We used data from the solute addition to calculate NH_4-N uptake length (the length an NH_4^+ ion travels downstream before biotic uptake), uptake velocity (the velocity at which biotic uptake removes N from the water column), and areal uptake rate (rate of biotic N uptake per unit area of streambed; Appendix 1C). Short-term nutrient releases can underestimate demand of

algae and microbes (Mulholland et al. 2002). To minimize this potential source of error we kept N addition low (2 – 5 times ambient NH4-N concentrations) while still allowing for detection at downstream transects.

Tests for significance of riparian canopy effect across streams were conducted using a mixed effects ANOVA model, with canopy cover as a fixed effect and stream as a random effect. Tests of canopy effects within individual streams were conducted using Student's *t* (SAS Institute, 2009). Data on *T. granifera* biomass density and N excretion rates were $\log_{10}$-transformed to satisfy ANOVA assumptions of normality and equal variance.

RESULTS

Food quantity, measured as chlorophyll *a* and AFDM, was significantly greater in open canopy habitats (chlorophyll *a*: $F_{1,28} = 33.52$, $p < 0.001$; AFDM: $F_{1,28} = 18.84$, $p < 0.001$; Appendix 1B). Within-stream comparisons indicate that algal biomass was greater in RAM (chlorophyll *a*: $t_{1,8} = 9.05$, $p < 0.001$; AFDM: $t_{1,8} = -4.14$, $p < 0.003$) and YAR (chlorophyll *a*: $t_{1,8} = 4.89$, $p < 0.001$; AFDM: $t_{1,8} = -4.72$, $p < 0.002$) but not ARI. Measurements of food quantity in intermediate canopy sites in RAM did not differ from open canopy habitat for chlorophyll *a*, and fell between open and closed canopy habitats (not significantly different from either) for AFDM. This suggests that light is saturating at intermediate levels of canopy cover. Food quality, as measured by molar C:N ratios, did not differ among canopy types in RAM and ARI, but was greater where canopy was open in YAR ($t_{1,8} = -3.73$, $p < 0.01$; Appendix 1B). Elemental analysis of *T. granifera* body C:N and C:P content (shell removed) in RAM and ARI showed no difference among canopy types (Appendix 1D).

Average snail density ranged from 23 individuals/m^2 (ARI closed canopy,

2008) to 1424 individuals/m^2 (RAM intermediate canopy, 2010). Canopy type influenced *T. granifera* biomass across the three study streams ($F_{1,38} = 22.06$, $p < 0.001$), and snail size distributions varied among streams (Figure 1.1). Within-stream comparisons indicated that snail biomass was greater in open canopy habitats in all streams, ranging from 2 to 8 times the densities found in closed canopy habitats (RAM: $t_{1,10} = -4.84$, $p < 0.001$; ARI: $t_{1,14} = -2.16$, $p = 0.047$; YAR: $t_{1,10} = -2.67$, $p = 0.028$; Figure 1.1). In 2010, no open canopy sites remained in the RAM study site due to riparian vegetation growth; however, snail densities exhibited near-significant differences among intermediate (mean $\pm$ 1 SE = 1424 $\pm$ 357 indivduals/m^2) and closed canopy (mean $\pm$ SE = 578 $\pm$ 218 indivduals/m^2) habitats ($t_{1,10} = -2.02$, $p = 0.07$). Nitrogen excretion rates of individual snails ranged from 0.02 to 0.18 umol N/h. Mass-specific excretion of individual snails decreased with increasing snail size and ranged from 0.001 to 0.05 umol N/mg AFDM/h. Snail N excretion showed allometric size scaling and differed significantly among canopy types (Figure 1.2; $F_{1,141} = 86.9$, $p < 0.001$). Within-stream comparisons indicated that snails in open canopy habitat exhibited greater excretion rates than closed canopy snails in RAM and ARI (RAM: $t_{1,60} = 5.73$, $p < 0.001$; ARI: $t_{1,37} = 2.07$, $p = 0.048$), but not YAR. Biomass-specific growth rates of *T. granifera* measured in a reciprocal transplant experiment were also greater in open canopy habitat regardless of treatment (control or transplanted; $F_{1,89} = 39.82$, $p < 0.001$). Response ratios of growth rate were similar for both transplants (0.74 for open to closed canopy, 0.68 for closed to open canopy (Figure 1.3). Confidence intervals (95%) did not cross zero, indicating a strong impact of canopy type on snail growth rates. Small snails grew faster than large snails across all treatments ($F_{1,89} = 26.77$, $p < 0.0001$) and initial snail size (before incubation) explained 23% of the variation in growth rates for the entire data set.

Areal excretion rates of *T. granifera* in 2008 (RAM, ARI, and YAR) ranged

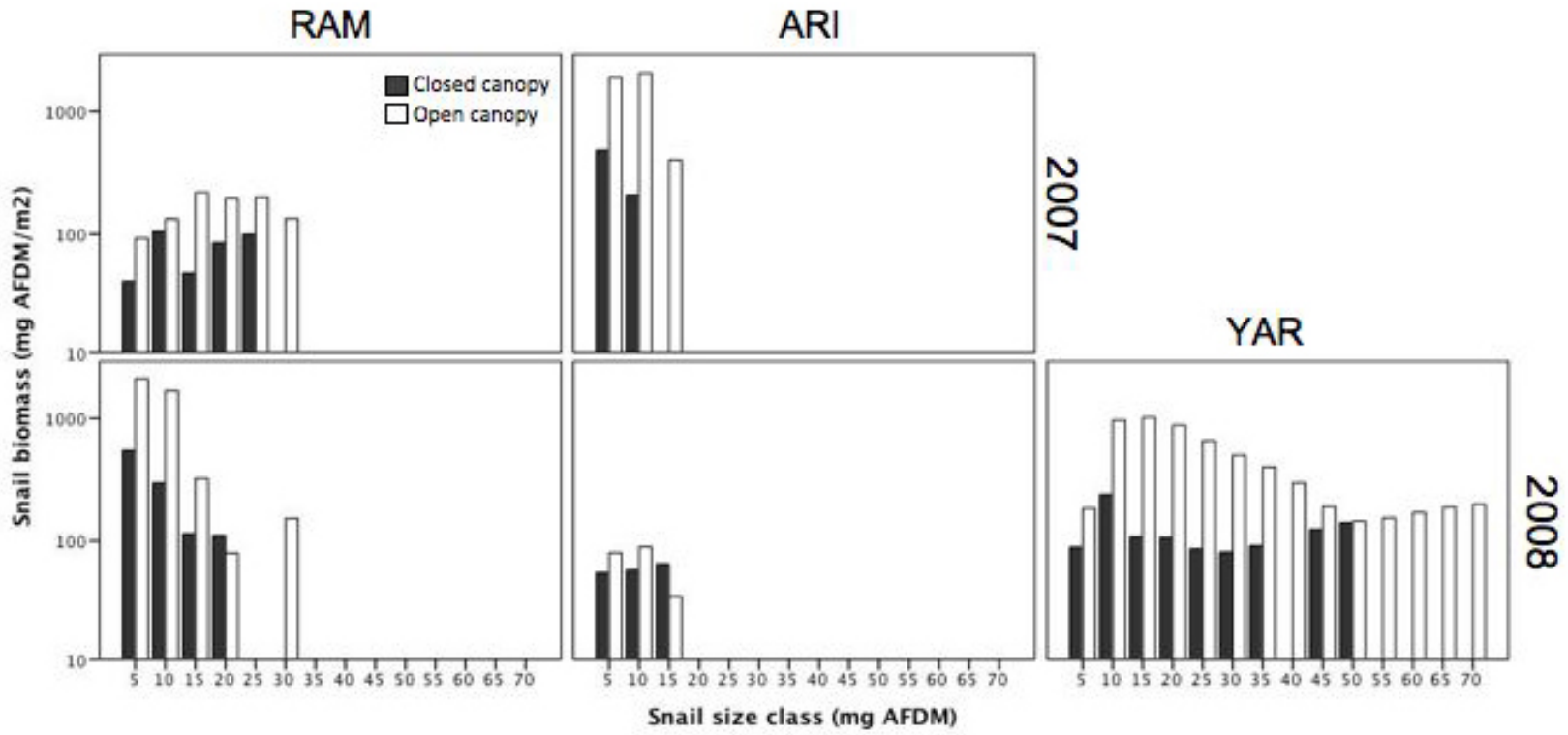

Figure 1.1. Mean *T. granifera* biomass by size class. Top row of panels are 2007 data and bottom row are 2008 data. Size classes were based on 5 mg AFDM increments. RAM = Ramdeen Stream, ARI = Aripo River, YAR = Yarra River. Gray and white bars represent data collected in closed and open canopy sites, respectively. Canopy type had a significant impact on *T. granifera* areal biomass ($F = 22.06$, $p < 0.0001$). Note log scale. No data were collected in YAR in 2007.

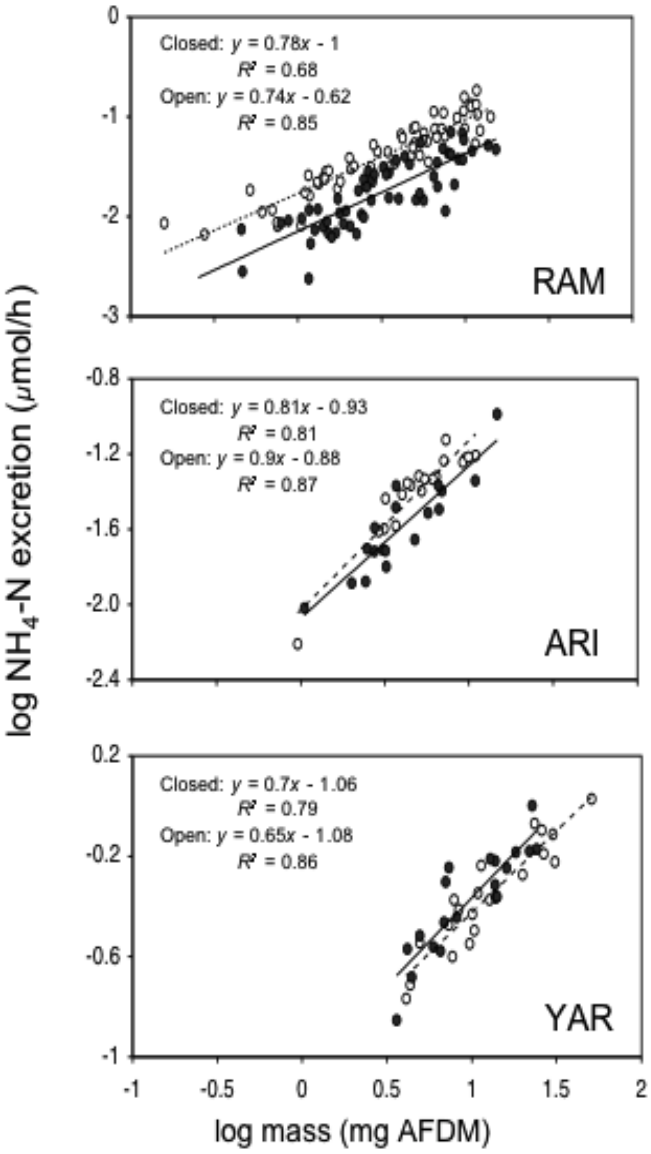

Figure 1.2. Size scaling of N excretion rates by *T. granifera* in Ramdeen Stream (RAM), Aripo River (ARI), and Yarra River (YAR). Open and closed circles represent individual snails collected in open and closed canopy habitats, respectively. Solid lines represent trends in closed canopy habitats, and broken lines represent trends in closed canopy habitats. Excretion was measured as moles of NH_4-N per hour. Mass was measured in mg ash-free dry mass. All data were log-transformed.

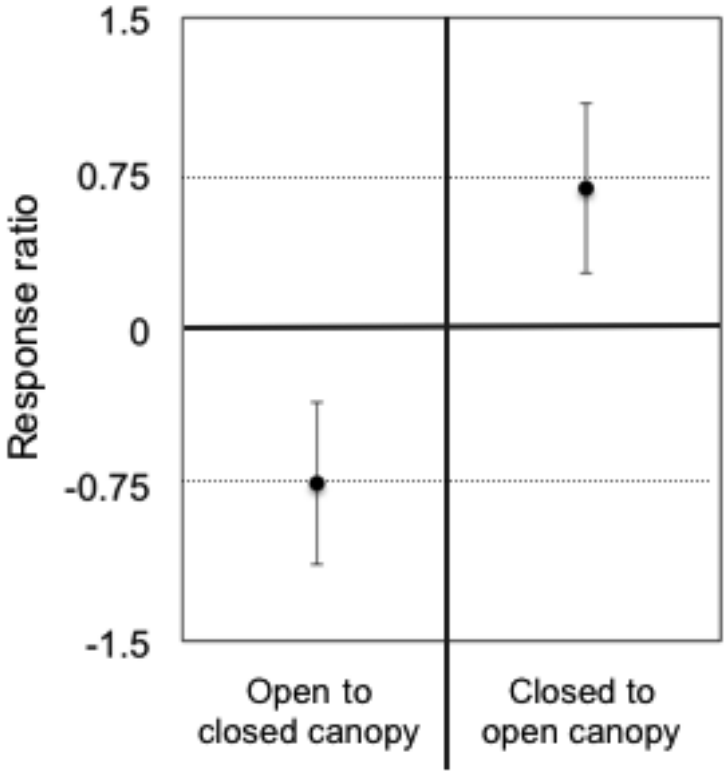

Figure 1.3. Effect sizes of canopy type on snail growth rates as measured in a reciprocal transplant experiment. Error bars represent 95% confidence intervals and effect sizes were measured as response ratios (Hedges et al. 1997). "Open to closed canopy" represents snails collected from open canopy habitat and moved to closed canopy habitat where growth rates were measured after 10 days, and "closed to open canopy" is vice versa.

from 0 to 900 ug N/m^2/h and were 3 to 9 times greater in open relative to closed canopy habitats. Differences in areal N excretion across canopy types for all three streams were significant (Figure 1.4, $F_{1,38} = 19.4$, $p < 0.0001$); within-stream analyses indicated areal excretion rates were greater in open canopy habitat across all streams (RAM: $t_{1,14} = -6.36$, $p < 0.001$; ARI: $t_{1,10} = -2.52$, $p = 0.025$; YAR: $t_{1,10} = -3.41$, $p < 0.01$).

Estimates of NH_4-N uptake rate in RAM in 2008 and 2010 from NH_4 release data showed a significant decline in background-corrected NH_4-N with distance downstream from the nutrient addition site ($R^2 = 0.75$ and 0.99, respectively; Appendix 1C). Using these data we calculated NH_4-N uptake length (2008: 75.8 m; 2010: 25.1 m), uptake velocity (2008: 5.7 mm/min; 2010: 10.2 mm/min), and area-specific uptake (2008: 4.19 mgN/m^2/h; 2010: 7.18 mgN/m^2/h; Appendix 1C). Mean areal NH_4-N excretion rates supplied 2% of areal NH_4-N demand in closed canopy habitat (in both 2008 and 2010), 11% of demand in intermediate canopy (2010), and 16% of areal NH_4-N demand in open canopy habitat (2008).

DISCUSSION

Our results suggest that invasive snails were heterogeneously distributed along streams due to differences in light availability. Human mediated removal of riparian vegetation can therefore have the unanticipated effect of creating hotspots of invasive snail excretion and influence on aquatic N cycling. The greater impact of *T.granifera* in open canopy sites was linked to increased snail density and, to a lesser extent, increased mass-specific excretion rates relative to shaded sites. Studies in temperate areas have shown that invasive mollusks can alter nutrient cycles in aquatic systems (Arnott and Vanni 1996, Hall et al. 2003); here, we have shown that the potential of a

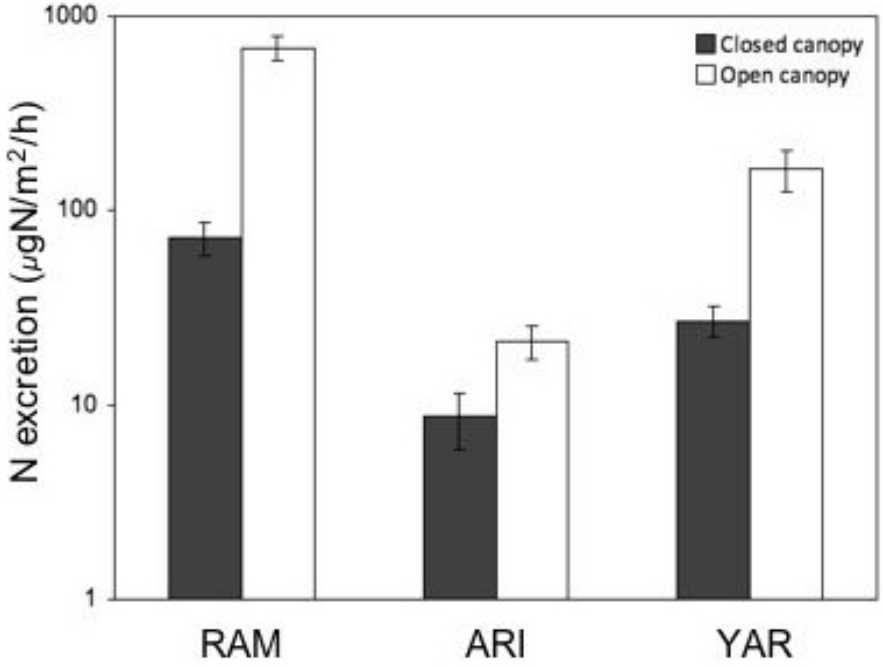

Figure 1.4. Mean (±1 SE) areal N excretion by *T. granifera* in 2008. RAM = Ramdeen Stream, ARI = Aripo River, YAR = Yarra River. Closed and open bars represent data collected in closed and open canopy sites, respectively. Canopy effect is significant across all streams ($p < 0.0001$). Note log scale.

single invasive species to impact N cycling in tropical streams is modified by the removal of vegetation in adjacent riparian zones.

Differences in snail impact among sites with different canopy cover were likely driven by variation in biomass of aquatic primary producers. In two out of the three study streams, estimates of algal and organic matter standing crop were significantly greater in open canopy sites where direct sunlight reaches the streambed and primary producers may be released from growth constraints posed by light limitation (Appendix 1B). In the Aripo River, estimates of food quantity did not differ among canopy types, perhaps due to unusually high rainfall in the 2008 dry season. The Aripo River is the largest of the three study streams, and intense scouring in this river relative to the other two study streams could have eroded spatial structure in algal production (Appendix 1B, Figure 1B). Increased light availability was more often associated with quantity than quality of snail food resources. Yarra River was the only study stream in which food quality was significantly greater in closed canopy habitat, and interestingly, the only stream for which the effect of canopy type on mass-specific excretion rates was not significant. In this stream, increased algal quality in closed canopy sites may have diminished the benefits of open canopy habitat where food was more plentiful but of poorer quality. Our results are consistent with studies linking invertebrate production and food resource quantity (Peterson et al. 1993, Wallace et al. 1999). In a landscape in which riparian zones are heterogeneously deforested, increased food availability in open canopy sites may act as spatial resource subsidies, driving increases in snail densities that "spill over" to less suitable closed canopy sites (Rand et al. 2006). It seems probable that patterns of riparian deforestation and associated increases in aquatic primary production may facilitate other herbivorous invasive species.

Invasion by *T. granifera* likely has altered ecosystem functioning and

community structure in tropical streams within our study area, though more data are necessary to understand the complexity of impacts. Lack of preinvasion datasets or comparable reference sites uninvaded by snails precluded direct analysis of ecosystem-level effects of invasion. However, results from this study have shown that primary production in Trinidadian lotic systems can be co-limited by availability of N and phosphorus. Changes in absolute and relative availability of these nutrients mediated by invasive snail excretion may influence which nutrient limits primary productivity, as well as the community composition of primary producers (Vanni 2002). These impacts on basal resources may disrupt food web dynamics, with unknown consequences on aquatic consumers. In addition, since few predators have been observed to consume *T. granifera* in Trinidadian streams despite dense and conspicuous aggregations of snails (S.B. Snider, *unpublished data*), these primary consumers may act as a trophic dead end for aquatic communities, diverting energy fixed by primary producers away from higher trophic levels (Wootton et al. 1996).

Snail size distributions influenced aggregate N excretion among study streams. Small-bodied organisms generally exhibit greater mass-specific excretion rates due to higher metabolism relative to large-bodied organisms (Hall et al. 2007). Differences in snail size distributions among our study streams strongly influenced areal excretion rates, as smaller snails excreted relatively more per unit mass than larger snails. In Ramdeen Stream, the snail size distribution was characterized by a high proportion of small individuals, whereas the Yarra population contained a high proportion of large snails (Figure 1.1). The difference in size structure led to increased areal excretion rates in Ramdeen open canopy habitat despite greater total snail biomass in Yarra (Figure 1.3).

Temporal dynamics likely moderate the impact of invasive snails on N cycling. This study was conducted during the dry season in the Caribbean, when stream

discharge is relatively low and flooding events typically infrequent. These conditions facilitate growth of primary producers that provide food sources for *T. granifera*. If snail biomass is generally associated with food quantity as our results suggest, areal snail excretion rates will be more pronounced during the dry season. In contrast, hydrologic variability due to frequent rain events in the wet season can dislodge snails, reducing snail biomass and eroding spatial structure by washing individuals downstream. The influence of riparian canopy is likely diminished in the wet season, as frequent scouring and streambed movement can continually reset algal growth on benthic substrate.

Understanding direct links between impacts of species introduction and habitat degradation is important for prioritizing use of limited funding for control efforts. Eradication—if possible—is often arduous and costly, and careful examination is necessary to identify the most urgent cases that have potential for success. Habitat degradation can unintentionally create patches of favorable habitat that act as spatial resource subsides to invasive species at landscape scales. Our study suggests that introduced populations of *T. granifera* can influence nutrient cycling in tropical systems, but mitigation efforts focused on curtailing habitat degradation in adjacent riparian corridors may ameliorate the magnitude of impacts. In this case, a management strategy involving restoration, protection of riparian buffer zones along stream corridors, or both, may decrease biogeochemical impacts of an invasive species. We suspect that direct links between habitat degradation and introduced animals alter nutrient cycles in other systems, and further investigation may uncover pragmatic management opportunities.

APPENDIX 1A

Nutrient diffusing substrates (NDS) were constructed following methods in Tank et al. (2006) and placed in RAM in 2007. NDS were constructed using 30 ml plastic canisters filled with agar gel amended with nutrient salts. The NDS analysis consisted of four nutrient treatments: a control (no nutrients added), nitrogen (N) added, phosphorus (P) added, and both N and P added. N was added in the form of NH_4NO_3 and P was added in the form of KH_2PO_4 to reach a concentration of 0.5 M for each compound. The agar gel regulated diffusion of nutrients through a fitted glass disc (glass crucible covers, Leco Corporation, St. Joeseph, Michigan, USA) that acted as a growth surface. The fritted glass disc was held in place by a tight fitting cap with a 2.2 cm diameter circular hole to expose the growth surface to the water column. Substrates were attached in a random sequence to PVC bars (length = 30cm; two bars of 12 NDS each, six total replicates for each treatment) and bars were secured to the stream bottom using metal stakes. Substrates were collected after an in-stream incubation period of two weeks and algal accrual was quantified as concentration of chlorophyll *a*. Glass discs were removed and extracted in a 90% buffered ethanol solution for 24 h and the concentration of chlorophyll *a* in the solution was quantified using a Turner Designs Aquafluor fluorometer (Sunnyvale, CA, USA) following methods in Arar and Collins (1997).

For statistical analyses of nutrient limitation, we used a randomized block ANOVA, with nutrient treatment as a fixed effect and bar number to which canisters were attached as a random effect followed by Tukey's HSD multiple comparison tests (SAS Institute, 2009). Results revealed that algal accrual was co-limited by N and P availability, as substrates containing both N and P were the only treatment with significantly greater algal biomass than controls ($P < 0.001$, Fig. 1A).

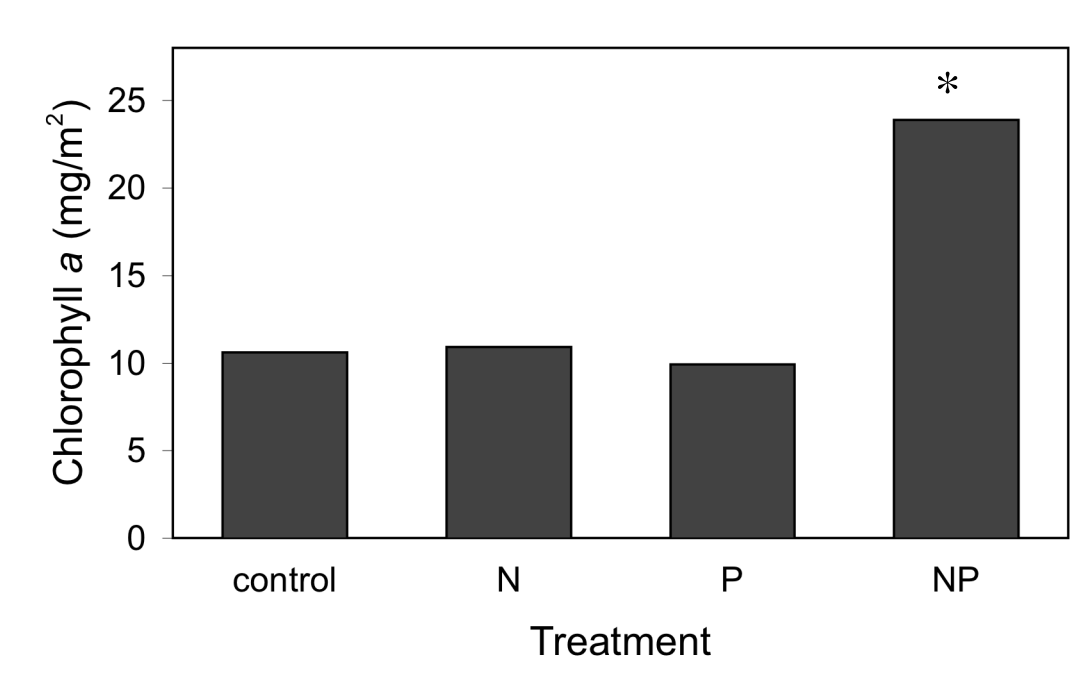

Figure 1A. Mean (±1 SE) chlorophyll *a* on nutrient diffusing substrates after a two-week incubation in RAM. Asterisk indicates significantly greater chlorophyll *a* relative to controls ($p < 0.001$).

APPENDIX 1B

Epilithon samples were collected in both open and closed canopy sites (5 total sites for each canopy type). Epilithon was collected by scrubbing the entire surface area of rocks on the stream bottom with a plastic bristle brush, generating a slurry of organic and inorganic matter (3 to 5 rocks scrubbed per slurry, 5 slurries per site). A subsample of known volume was removed from the slurry using a pipette and filtered (Pall-Gelman, Type A/E 25mm or 47 mm) for chlorophyll *a* (as an indicator of algal biomass) and ash-free dry mass (AFDM; as an indicator of organic material biomass) analyses. Upon filtration, chlorophyll *a* samples were extracted in 90% buffered ethanol for 24 hours and analyzed using fluorometric techniques (Arar and Collins 1997; Turner Designs Aquafluor, Sunnyvale, California, USA). AFDM samples were processed following methods in Wallace et al. (2006). To generate areal estimates of algal biomass and AFDM, we traced rocks used for each slurry and calculated areas of tracings using ImageJ software (National Institutes of Health, Bethesda, Maryland, USA). The remaining slurry volume was settled in refrigerated conditions over a 24-h period after which water was decanted and the remaining concentrated sample dried in a drying oven at 55 °C for subsequent elemental composition analysis at Cornell University. Once transported to the laboratory, samples were homogenized and weighed on a microbalance (Mettler Toledo MX5, Columbus, OH, USA) to the nearest ug. C and N content of samples were analyzed using an elemental analyzer (Elementar Vario EL III, Frankfurt, Germany). Chlorophyll *a* and AFDM were significantly greater in open canopy habitats (chlorophyll *a*: $F_{1,28} = 33.52$, $p < 0.001$; AFDM: $F_{1,28} = 18.84$, $p < 0.001$, Figure 1B).

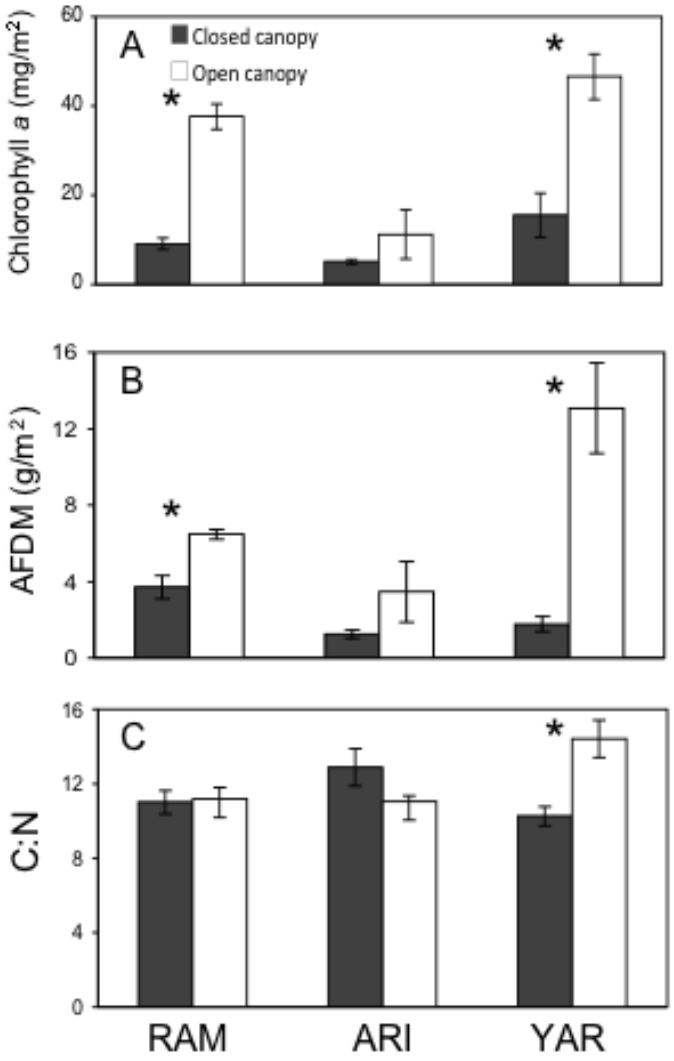

Figure 1B. Mean (±1 SE) chlorophyll *a* (A), ash-free dry mass (AFDM) (B), and molar C:N ratios of epilithon (C). RAM = Ramdeen Stream, ARI = Aripo River, YAR = Yarra River. Gray and white bars represent data collected in closed and open canopy sites, respectively ("closed" ≥ 75% and "open" ≤ 25% canopy cover). Asterisks above bars represent significant differences ($p < 0.05$) among canopy types within streams.

APPENDIX 1C

Uptake length, or average distance a NH_4 molecule travels downstream before it is taken up by biota, was calculated using the exponential decay model $\ln N_x = \ln N_0 - kx$, where N_0 and N_x are background-corrected NH_4 concentrations at the addition site and x meters downstream from the addition site, k is the exponential decay rate, and uptake length is $1/k$ (Newbold et al. 1981; $p < 0.01$, Figure 1C). Uptake length measurements vary with stream discharge as greater discharge will carry nutrient molecules further before they make contact with the streambed and are incorporated by biota. To control for variation in discharge we calculated NH_4 uptake velocity, or NH_4 demand relative to NH_4 concentration in streamwater, as V_f (m/min) = Qk/w where Q is discharge (m^3/min), k is the inverse of uptake length, and w is mean stream width. We used the uptake velocity estimate to calculate areal uptake rate as U ($mgN/m^2/h$) = $V_f N_b$ * 60min/h, where N_b is mean background NH_4 concentration (n = 10) before solutes were added to the stream (Newbold et al. 1981).

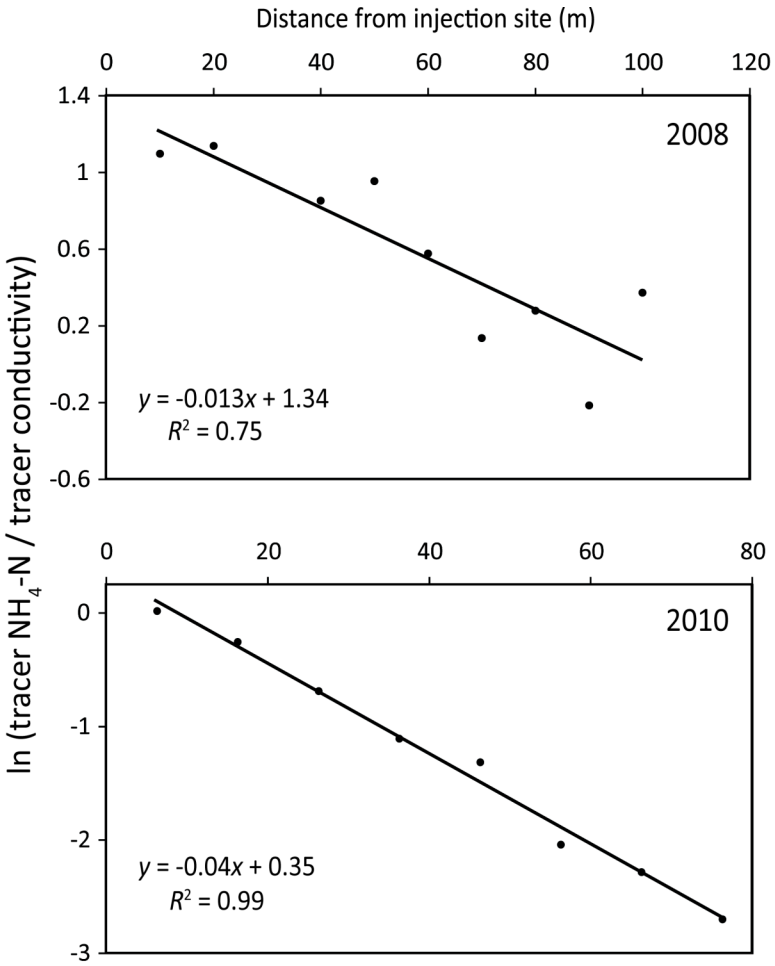

Figure 1C. Short-term NH_4 addition in RAM in 2008. Tracer NH_4-N and conductivity are concentrations at plateau corrected for background concentrations. Distance from injection site indicates location downstream from site where solutes were added using a peristaltic pump.

APPENDIX 1D

For elemental composition analysis of snail body tissue, shells were removed and body tissue dried to a constant weight, ground to a fine powder, and homogenized using a mortar and pestle. Subsamples were weighed on a microbalance (Mettler Toledo MX5) to the nearest μg and analyzed for C and N content using a CHNS elemental analyzer (Elementar Vario EL III). For P content, subsamples were weighed into acid-washed Pyrex tubes, ashed at 500 °C, digested in 1N HCl, and analyzed on a Shimadzu UV 1240 spectrophotometer (molybdate blue method, Murphy and Riley 1962). Ground citrus leaves and spinach (US National Institute of Standards and Technology, US Department of Congress) were used as standards for P analysis and were analyzed in each set of samples. Percent recovery of P from standards was typically 97-100%. Elemental ratios are molar.

Table 1D. Mean (± 1SE) body tissue C:N and C:P of *T. granifera* (shell removed) in open and closed canopy habitat. RAM = Ramdeen Stream, ARI = Aripo River.

	RAM		ARI	
	C:N	C:P	C:N	C:P
Closed canopy	5.3 ± 0.3	114 ± 9	6.0 ± 0.3	93 ± 3
Open canopy	5.4 ± 0.3	116 ± 10	5.5 ± 0.2	104 ± 5

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CHAPTER 2

TOWARDS DEFINING STOICHIOMETRIC TEMPLATES AT REGIONAL SCALES: CONSUMER-RESOURCE ELEMENTAL COMPOSITION IN A HIGH ELEVATION STREAM NETWORK

ABSTRACT

The ability to predict when and where food quality constrains ecological interactions often hinges on identification of factors that influence the chemical composition of consumers and their food resources. We measured carbon (C), nitrogen (N), and phosphorus (P) content of benthic consumers and basal resources across a sub-alpine stream network to determine spatial variability in elemental composition of food web components and mismatches in consumer-resource C:N:P stoichiometry. In addition, we measured physical, chemical, and biological characteristics of streams to identify key factors that best explained stoichiometic variation patterns. We found that basal resources across the sub-alpine stream network had high N and P content compared to other lotic systems, despite widespread P limitation of algal accrual. For benthic consumers, functional feeding groups differed in elemental composition but individual taxa did not differ among streams, supporting the notion that consumers are homeostatic relative to primary producers. Elemental imbalances between basal resources and primary consumers were small for N content, and basal resources had greater P content relative to consumers. These findings revealed a limitation decoupling between benthic epilithon (primarily P limited) and primary consumers (C and N limited). Physical characteristics of streams (size, temperature, and disturbance regime) best described variability in epilithon nutrient content at the regional scale, and were therefore the factors most likely to influence elemental mismatches between epilithon and consumers. None of the measured nutrient characteristics (dissolved inorganic N and P, magnitude of P limitation) and only one biological characteristic (mayfly density; but not caddisfly density, predatory invertebrate density, fish density) were significant predictors of epilithon nutrient content. Our results suggest that food quantity may be a more important driver of consumer dynamics than food quality in

some sub-alpine stream networks.

INTRODUCTION

Identifying key factors that influence the chemical composition of consumers and their resources is necessary to predict conditions when and where food quality will impinge on ecological interactions. Ecological stoichiometry – the mass balance of chemical elements in ecological processes – has been increasingly used as a tool to identify resource quality constraints on consumer dynamics in benthic systems (Cross et al. 2003, Bowman et al. 2005, Frost et al. 2006, Hladyz et al. 2009). A tenet of ecological stoichiometry theory is that mismatches between elemental ratios [most often carbon : nitrogen : phosphorus (C:N:P) ratios] of consumers and resources can be used to infer the relative importance of food quantity versus quality in driving consumer dynamics (Sterner 1997, Sterner and Elser 2002). Future efforts to mitigate environmental change such as eutrophication, climate change, and land use conversion will depend in part on an improved ability to predict consequences for consumer-resource dynamics. Prediction of outcomes will be enhanced by identification of when and where consumer growth and reproduction are limited by energy, nutrient acquisition, or both (e.g. Sterner 1997, Allen and Gillooly 2009), which necessitates additional information on factors that describe variability in elemental composition of food web components and mismatches between consumers and their food resources.

Ecological stoichiometry theory pertaining to food quality versus quantity constraints has been most often empirically tested in freshwater pelagic systems, although the framework has been useful for studies of terrestrial (Fagan and Denno 2004) and aquatic benthic systems (Cross et al. 2003, Evans-White et al. 2005, Hladyz et al. 2009). Stoichiometic studies in alpine and sub-alpine streams are particularly

sparse, despite their distinct abiotic attributes (e.g. freeze-thaw regimes, truncated growing seasons, increased importance of autochthonous organic matter) and the acute vulnerability of high-elevation biota to climate change (Hauer et al. 1997, McCarthy et al. 2001). Changes in precipitation and temperature regimes are likely to have substantial effects on species distributions and interactions in these systems (Hauer et al. 1997), yet our baseline knowledge of the causes and consequences of variable elemental composition of alpine and sub-alpine food webs is limited. Information on the biotic and abiotic factors that control variability in elemental composition of benthic food web components (i.e. the "stoichiometric template" *sensu* Schade et al. 2005) will enhance our capacity to identify appropriate predictors for stoichiometric models, and by extension, better understand whether consumer growth is limited by food quality or quantity (Frost et al. 2002b).

Previous studies have shown that food quality can limit consumer growth rates in lentic and lotic systems (Elser et al. 2000, Boersma and Kreutzer 2002, Frost and Elser 2002b, Stelzer and Lamberti 2002). Recent work has begun to unravel the mechanisms that influence the elemental composition of food resources and consumers in streams, and thus determine the likelihood and degree of food quality limitation. In benthic systems, research has demonstrated that the elemental content of basal resources is influenced by both biological and chemical pathways; for example, the presence of invertebrate grazers increased N and P content of epilithon via consumption and excretion (Hillebrand and Kahlert 2001, Frost et al. 2002a, Evans-White and Lamberti 2006, Hillebrand et al. 2008) and dissolved inorganic nutrient availability was positively related to epilithon nutrient content (Stelzer and Lamberti 2001, Frost and Elser 2002a, Cross et al. 2003, Liess and Hillebrand 2006). Determining the identity of other key physical, chemical, and biological factors and their relative importance in streams will move us towards defining stoichiometric

templates of lotic ecosystems (Frost et al. 2002b, Schade et al. 2005).

To date, studies of the stoichiometric interplay between consumers and resources in lotic systems have largely focused on a small number of streams (but see Evans-White et al. 2005) and have not simultaneously measured physical, chemical, and biological variables to assess potential predictors of stoichiometric patterns. This spatially-constrained approach is useful for comprehensive studies of food web stoichiometry within select streams, but precludes the ability to determine spatial and temporal variability within and across watersheds. Other regional conceptual frameworks have highlighted the importance of longitudinal and spatial heterogeneity in streams (Vannote et al. 1980, Townsend 1989) and addressed the importance of temporal variability associated with hydrologic and geomorphic disturbance regimes (Townsend et al. 1997). In taking a regional approach to describing patterns in stream stoichiometry, the inherent variability in stream systems can be used to identify and quantify characteristics that shape stoichiometric templates and provide baseline information useful for predicting when and where elemental composition of resources and consumers will be important drivers of food web dynamics.

In this study, we quantified variability in consumer-resource stoichiometry across a sub-alpine stream network and identified the physical, chemical, and biological characteristics of streams that best explain stoichiometric variation. We hypothesized that conditions favorable to species with fast growth rates (e.g. frequent disturbance, high predation) would likely be associated with relatively high P demand due to high ribosome (a P-rich organelle) concentration in tissues of fast-growing organisms (growth rate hypothesis, Sterner and Elser 2002). In addition, streams with relatively higher nutrient availability (dissolved inorganic N and P) would support benthic producers with correspondingly lower C: nutrient ratios. The increased nutrient content of basal resources was hypothesized to reduce elemental imbalance

between producers and primary consumers, based on evidence from previous studies (Cross et al. 2003, Bowman et al. 2005) in which dissolved nutrient enrichment led to subsequent decreases in C:N and C:P ratios of epilithon. Consumers, in contrast to producers, were expected to exhibit relatively homeostatic chemical composition regardless of the chemical composition of their food (Elser et al. 2000, Sterner and Elser 2002). With this study approach, we were also able to compare the stoichiometric profile of high-elevation streams to previously reported values of other systems and reveal patterns of stoichiometric variability across a stream network.

METHODS

Study sites

To determine the spatial variability of elemental composition within benthic food webs, we conducted a field survey in 50-m reaches of 10 streams in the East River drainage basin near the Rocky Mountain Biological Laboratory (elevation 2900 m) in western Colorado, USA (Table 2.1). Much of the catchment consists of sub-alpine spruce-fir-aspen forests, and riparian willows often border streams. Streams are either snowmelt- or spring-fed, and although timing of hydrologic disturbance events is predictably tied to spring snowmelt, the magnitude and frequency of disturbance is variable across streams (B. Peckarsky and A. McIntosh, *unpublished data*). Bed substrates are stony and epilithic diatoms dominate primary producer communities (although bryophytes and filamentous algae are often present at some sites). Invertebrate communities are dominated by Ephemeroptera (mayflies), Tricoptera (caddisflies), Plecoptera (stoneflies), and Diptera (true flies). Five of the streams contain populations of brook trout (*Salvelinus fontinalis*), and the remaining 5 are fishless due to natural barriers to fish dispersal. Streams also vary moderately with

respect to dissolved nutrient availability (Table 2.1).

Stream characterizations

Physical variables.

To characterize physical attributes of streams we measured stream width, discharge, temperature, and substrate particle size distribution. We posited that disturbance could play a significant role influencing elemental ratios of consumers and resources; thus we also quantified four measures of disturbance. The first two disturbance measures quantified the total number of hours stream discharge was 80-100% of bankfull stage, either for (1) the water year in which consumer-resource stoichiometry samples were collected (2006-2007), or (2) a three-year period (2005-2008). Bankfull discharge was chosen because it is the dominant channel forming flow (Allan and Castillo 2007). The other disturbance measures included a theoretical index of substrate movement due to the force exerted by water flow per unit area (Shields' stress, Shields 1936; e.g. Wilcox et al. 2008) and a subjective composite measure of habitat disturbance that involves scoring channel variables along the upper bank, lower bank, and stream bottom (Pfankuch's index, Pfankuch 1975; e.g. Townsend et al. 1997). Continuous discharge for all 10 streams was calibrated from stage height data loggers (TruTrack model WT-HR, Christchurch, New Zealand) for three years (2005 – 2008). Loggers also collected continuous water temperature data. Shields' stress (τ^*) – the critical shear stress required to move a particle of a given size – was calculated as:

$$\tau^* = \tau_o / (\rho_s - \rho_w) g D_x$$

where τ_o is boundary shear stress, calculated as:

$$\tau_o = \rho_w g R S$$

ρ_s is substrate density, ρ_w is water density, g is gravitational acceleration, R is

hydraulic radius, S is slope (channel gradient), and D_x is representative grain diameter (in this case the median particle size). Random-walk pebble counts (Wolman 1954) were conducted in each study reach to measure particle size distributions and to calculate median particle size. Hydraulic radius was obtained by measuring the cross-sectional area (A) and wetted perimeter (P) of each stream channel at three randomly chosen sites ($R = A / P$).

Chemical variables.

Filtered water samples (Pall-Gelman A/E filters) were collected in each stream in June and August of 2006 and 2007. A subset of samples was immediately analyzed for NH_4 using a Turner Designs Aquafluor (Sunnyvale, CA) following fluorometric methods in Taylor et al. (2007), and the remainder were frozen for subsequent analysis of NO_3 using an ion chromatograph (Dionex ICS-2000) and soluble reactive phosphorus (SRP) using a spectrophotometer (Shimadzu model 1240; molybdate blue method, Murphy and Riley 1962).

We used nutrient diffusing substrates (NDS) to test whether nutrient availability limited algal biomass accrual in 2006 and 2007. Substrates were constructed using 30 ml plastic canisters filled with a water-based solution amended with nutrient salts. The NDS analysis consisted of four nutrient treatments: a control (no nutrients added), nitrogen (N) added, phosphorus (P) added, and both N and P added. N was added in the form of NH_4NO_3 to reach a concentration of 200mM, and P was added in the form of KH_2PO_4 to a concentration of 8 mM to achieve a dissolved inorganic N to dissolved inorganic P ratio that fell within the range of previously reported values for the East River drainage basin (Peckarsky et al. 2001) and within the range of values observed in this study (Table 2.1). A nylon membrane (GE Magna, 0.022 um) was used to regulate diffusion of nutrients through a glass fiber

filter (Pall-Gelman A/E) that acted as a growth surface (method modified from Matlock et al. 1998 and Tank et al. 2006). The nylon membrane and glass fiber filter were held in place by a tight fitting cap with a 2.2 cm diameter circular hole to expose the growth surface to the water column. Substrates were attached in a random sequence to PVC bars (length = 30 cm; two or four bars of eight NDS each, two replicates of each nutrient treatment per bar) and bars were secured to the stream bottom using metal stakes. After seven days of incubation in the stream, NDS were briefly removed, filled with new nutrient solution while shaded, and redeployed for an additional seven days for a total of 14 days of in-stream incubation. Adding new nutrient salt solution was necessary because plastic canisters of larger volume could not be adequately submerged in the smaller study streams. Preliminary diffusion tests determined that NDS of the size used in this study would diffuse sufficiently for a period of seven days but a total of 14 days was necessary for sufficient algal growth (J. Moslemi, *unpublished data*). At the end of the NDS experiment, algal accrual was quantified as concentration of chlorophyll *a*. Glass fiber filters were removed and extracted in a 90% buffered ethanol solution for 24 h and the concentration of chlorophyll *a* in the solution was quantified using a Turner Designs Aquafluor fluorometer following methods in Arar and Collins (1997).

Biological variables

To characterize biological attributes of streams, we quantified epilithon (as ash-free dry mass, AFDM), invertebrate density, and fish density. AFDM was quantified using an open-ended plastic cylinder (circular area = 0.05 m^2); three replicates were collected at random locations in each study reach in 2006. To estimate fine particulate organic matter and mass of epilithon, the plastic cylinder was firmly placed in the streambed and five water depths within the cylinder were recorded for

calculations of total cylinder water volume. Within the cylinder, rocks were brushed and sediments vigorously agitated to a depth of about 5 cm and the slurry was subsampled and filtered onto a pre-ashed, pre-weighed glass fiber filter (Pall-Gelman A/E). In the laboratory filters containing sample material were dried at 60 °C, weighed, combusted at 500 °C, and reweighed to determine AFDM per unit area sampled.

Invertebrates were sampled at three random locations within each study reach using a custom-made box sampler (0.104 m^2 area, 360 μ mesh size) and preserved in 95% ethanol. In the laboratory, individuals were identified to lowest possible taxon (genus or species), and functional feeding groups (FFGs; i.e. grazer, shredder, predator) were determined. Taxa occupying more than one FFG were categorized as omnivores. To estimate invertebrate biomass, average weight of individuals for each taxon was calculated from samples taken for a separate study in the same drainage basin and multiplied by observed invertebrate densities. In streams where fish were present, fish densities were determined by three-pass depletion electrofishing (Smith-Root electrofisher, Model LR-24, Vancouver, WA. USA) between stop nets at ends of 50 m study reaches, and calculated using maximum likelihood equations (Cowx 1983; see McIntosh et al. 2002 for more details).

Consumer-resource stoichiometry

We sampled epilithon for stoichiometric analysis in each of the 10 study streams in July 2007. Epilithon was collected in three random locations within each study reach by scrubbing cobbles with a plastic bristle brush, generating a slurry of organic and inorganic matter. Slurries were filtered through a 500 μm sieve to remove macroinvertebrates and then allowed to settle before excess water was decanted. Due to the limited epilithon mass in cold, high-elevation streams, the three replicates

within each site needed to be pooled into a single sample for each stream. Samples were dried at 60 °C and stored in a desiccator until processed for elemental composition analysis.

Benthic macroinvertebrates were collected by hand or with D-nets within study reaches of each stream. The number of individuals sampled varied with the size of each taxon, ranging from two individuals for large taxa (e.g. *Megarcys signata* stoneflies) to tens of individuals for smaller taxa (e.g. *Baetis bicaudatis* mayflies). Individuals of each taxon were pooled by site to obtain sufficient material for elemental analysis. Macroinvertebrates were kept in vials with stream water for several hours to allow for clearance of guts, identified to the lowest taxonomic level possible (genus or species) using a dissecting microscope, and then frozen until further processed.

For elemental composition analysis, epilithon and macroinvertebrate samples were dried to a constant weight at 55 °C and then ground to a fine powder and homogenized using a mortar and pestle. Subsamples were weighed on a microbalance (Mettler Toledo MX5) to the nearest μg and analyzed for C and N content using a CHNS elemental analyzer (Elementar Vario EL III). For P content, subsamples were weighed into acid-washed Pyrex tubes, ashed at 500 °C, digested in 1N HCl, and analyzed on a Shimadzu UV 1240 spectrophotometer (molybdate blue method, Murphy and Riley 1962). Ground citrus leaves and spinach (US National Institute of Standards and Technology, US Department of Congress) were used as standards for P analysis and were analyzed in each set of samples. Percent recovery of P from standards was typically 97-100%. Total C, N, and P are presented as percentage of dry mass, and elemental ratios are molar.

Statistical analyses

To assess the magnitude of the response of algal biomass to limiting nutrients (i.e. phosphorus; see *Results*) in the NDS experiment, we determined effect sizes by calculating a limitation response index which was the log ratio of the nutrient treatment to the unenriched controls (i.e., phosphorus = log ([chlorophyll *a* on phosphorus-enriched substrata]/[chlorophyll *a* on control substrata]); see Flecker et al. 2002). For statistical analyses, we used a randomized block ANOVA, with nutrient treatment as a fixed effect and bar number to which canisters were attached as a random effect (SAS Institute, 2009). To determine variation in macroinvertebrate elemental composition among streams, a coefficient of variation (CV) for each single element and ratio was calculated for taxa present in at least three streams.

To assess the stream characteristics most strongly associated with nutrient content of basal food resources, relationships between epilithon and macroinvertebrate C, N, and P stoichiometry and physical, chemical, and biological variables were examined using simple and stepwise multiple linear regression (SLR and MLR, respectively) to determine the most efficient models. Predictors of epilithon and macroinvertebrate C, N, and P content (i.e. measures of hydrologic disturbance regime, stream size and other physical characteristics, dissolved nutrient availability, and density of grazers and predators) were tested for multicollinearity by regressing all potential predictors against each other and checking variance inflation factor (VIF) values. Non-orthogonal variables (i.e. VIF > 10) were excluded from the study (Myers 1986). SLR and MLR analyses were preformed using PASW software (version 18.0, Chicago, IL). We also obtained standardized regression coefficients (β_{std}) to assess the relative influence of predictors on nutrient content variables. β_{std} represents the change in the dependent variable in units of standard deviations that results from a change of one standard deviation of the corresponding independent variable (Neter et al. 1996). The magnitude of β_{std} thus indicates which variables have

the greatest effect on the predicted value. Where necessary, variables were log transformed to achieve linearity and approximately homogeneous distributions of residuals.

We compared body nutrient composition (%C, %N, %P, C:N, C:P, N:P by dry weight) among invertebrates using multivariate analysis of variance (MANOVA) to avoid experiment-wise error. We used Wilks' likelihood ratio test to determine MANOVA significance. Significant variables in MANOVA tests were subsequently analyzed with one-way ANOVAs followed by Tukey's HSD multiple comparison tests. We tested for variation among taxonomic groups, FFGs, and streams only for those taxa that were present in three or more study streams (PASW software, version 18.0).

RESULTS

Stream characterization

Study streams varied substantially in size and discharge, and moderately in temperature and dissolved inorganic nutrient availability (Table 2.1). Analysis of algal biomass on NDS revealed that algal accrual was largely limited by P availability; eight of the 10 study streams were P limited, and two were co-limited by N and P (Table 2.2). In the two co-limited streams (i.e. Marmot and B9), P addition increased algal accrual, but algal biomass was significantly greater than controls only when P was added in combination with N. The P limitation response index revealed consistent increases of algal biomass when P was added, though the relative degree of P limitation varied among streams (P limitation index range: 0.069 – 0.87, Table 2.1). In contrast, the addition of N alone did not significantly increase algal biomass relative to controls in any of the study streams (Table 2.2).

Table 2.1. Summary of physical and chemical characteristics of 10 study streams in the East River drainage basin. Data were collected in 2006.

Stream	Width (m)	Maximum discharge (m3/s)	Median substrate particle size (mm)	Temperature (°C)	DIN (ug/L)	SRP (ug/L)	Phosphorus limitation index
B2	0.9	0.034	33	-0.5-8.8	168	< 1	0.830
B3	1	0.004	24	0.1-8.6	166	1.9	0.609
B9	0.9	0.008	43	0.8-10.8	54	< 1	0.190
COP	8.9	5.370	70	-0.1-11.9	78	2.0	0.391
EST	10.7	4.602	105	-0.4-16.3	99	4.0	0.877
LBE	1.9	0.247	58	0.1-14.2	126	2.0	0.188
LBR	1.9	0.835	80	-0.1-12.8	148	3.7	0.467
MAR	0.9	0.045	44	-0.3-9.2	92	3.0	0.393
QUG	2.4	0.884	49	-0.2-10.5	142	4.1	0.567
UPE	3.5	1.289	59	-0.1-12.7	85	1.8	0.069

Notes: Abbreviations are: DIN = dissolved inorganic nitrogen, SRP = soluble reactive phosphorus. Temperature data represent ranges observed in 2006. Phosphorus limitation index was calculated from results of the NDS experiment (see "Statistical analyses" in Methods). Stream codes are as follows: B2 = Benthette Creek #2, B3 = Benthette Creek #3, B9 = Benthette Creek #9, COP = Copper Creek, EST = East River, LBE = Lower Benthette Brook, MAR = Marmot Creek, QUG = Quigley Creek, UPE = Upper East River

Table 2.2. Mean (± 1 SE) chlorophyll *a* on nutrient diffusing substrates and ANOVA models for regional analysis of algal nutrient limitation in 10 study streams. Letters represent significant differences within a given study stream. Posthoc tests were performed using Tukey's HSD, and degrees of freedom = 3 for all models.

Stream	*F*	*P*	Control	+N	+P	+N, +P	Limiting nutrient(s)
B2	35.73	<0.001	(16 ± 1.8)^{a}	(18 ± 3.1)^{a}	(35 ± 2.0)^{b}	(39 ± 3.2)^{b}	P
B3	17.12	<0.001	(17 ± 1.8)^{a}	(12 ± 1.1)^{a}	(31 ± 2.0)^{b}	(27 ± 1.9)^{b}	P
B9	5.59	0.096	(11 ± 1.4)^{a}	(11 ± 1.0)^{a}	(17 ± 2.5)^{a,b}	(21 ± 2.8)^{b}	NP co-limited
COP	26.65	<0.001	(26 ± 1.1)^{a}	(23 ± 2.8)^{a}	(40 ± 2.6)^{b}	(39 ± 1.6)^{b}	P
EST	6.72	0.011	(7.5 ± 2.2)^{a}	(7.0 ± 0.9)^{a}	(15 ± 2.1)^{b}	(18 ± 1.6)^{b}	P
LBR	13.54	0.001	(20 ± 2.5)^{a}	(21 ± 1.8)^{a}	(32 ± 2.4)^{b}	(34 ± 2.0)^{b}	P
LBE	4.65	0.016	(36 ± 1.6)^{a}	(40 ± 1.7)^{a,b,c}	(44 ± 1.5)^{b,c}	(44 ± 1.6)^{b,c}	P
MAR	1.98	0.187	(14 ± 1.4)^{a}	(16 ± 3.7)^{a}	(21 ± 1.4)^{a,b}	(25 ± 4.4)^{b}	NP co-limited
QUG	10.25	0.003	(11.3 ± 2.6)^{a}	(11 ± 2.0)^{a}	(18 ± 3.4)^{b}	(16 ± 2.7)^{b}	P
UPE	1.5	0.370	(37 ± 2.9)^{a}	(38 ± 2.0)^{a}	(43 ± 0.5)^{a*}	(43 ± 0.6)^{a}	P limitation*

Note: (*) indicates *P*-value for +P treatment = 0.058.

Epilithon stoichiometry

Epilithon nutrient content varied among streams (% C range = 5.9 – 15.1, % N range = 0.35 – 1.93, % P range = 0.10 – 0.21, Table 2.3). The nutrient content of benthic macroinvertebrates varied across taxa (Figures 2.1 and 2.2), but individual taxa found in several (5 to 10) study streams (*Baetis bicaudatis, Cinygmula* spp., *Kogotus modestus*, *Megarcys signata*) displayed few differences among streams. The among-stream CVs for nutrient content of macroinvertebrates found in at least three streams differed among taxa and FFGs (Figure 2.3). Phosphorus content typically exhibited the most variation among streams (*Parapsyche almota* is the one exception for which %N had greatest variability, Figure 2.3a), and CVs for nutrient ratios containing P (C:P and N:P) were generally higher than CVs for C:N (Figure 2.3b). Differences among FFGs were not significant.

Invertebrate stoichiometry

Macroinvertebrate taxa within a given FFG did not differ in elemental composition across streams (grazers: Wilks' $\lambda = 0.71$, $P = 0.85$; omnivores: Wilks' $\lambda = 0.62$, $P = 0.77$; predators: Wilks' $\lambda = 1.56$, $P = 0.23$). Omnivorous taxa differed in elemental composition (Wilks' $\lambda = 63.4$, $P = 0.002$), but grazing and predatory taxa did not (grazers: Wilks' $\lambda = 1.25$, $P = 0.26$; predators: Wilks' $\lambda = 1.80$, $P = 0.10$; Figures 2.1 and 2.2). Subsequent one-way ANOVAs for each single element and ratio revealed that omnivorous taxa differed in % N ($F_{3,5} = 6.0$, $P = 0.04$), % P ($F_{3,5} = 23.4$, $P = 0.002$), C:P ($F_{3,5} = 6.56$, $P = 0.04$), and N:P ($F_{3,5} = 35.0$, $P = 0.001$). Grazing taxa differed in % N ($F_{5,13} = 3.5$, $P = 0.03$; Figure 2.1) and predatory taxa differed in % N ($F_{3,12} = 17.3$, $P < 0.001$; Figure 2.1) and C:N ($F_{3,12} = 9.08$, $P = 0.002$; Figure 2.2). Comparisons of FFGs indicated that FFGs differed in their elemental composition (Wilks' $\lambda = 0.22$, $P < 0.001$; Table 2.4). Predators had the highest % N and % P,

Table 2.3. Comparisons of carbon (C), nitrogen (N), and phosphorus (P) contents, and C:N, C:P, and N:P ratios among epilithon and grazer communities (mean for all grazer taxa) in sub-alpine streams. Grazer-epilithon elemental imbalances are arithmetic differences between the trophic groups and all ratios are molar.

	Epilithon						Grazers						Grazer-epilithon imbalance		
Stream	%C	%N	%P	C:N	C:P	N:P	%C	%N	%P	C:N	C:P	N:P	C:N	C:P	N:P
B2	11.8	1.62	0.21	8.5	142	17	46.6	9.04	0.58	6.1	211	37	2.4	-69	-20
B3	8.9	0.71	0.12	14.7	190	13	53.4	8.99	0.55	6.9	232	34	7.7	-42	-21
B9	8.8	0.74	0.13	13.9	181	13	50.9	8.06	0.44	7.5	308	42	6.3	-126	-29
COP	15.1	1.93	0.17	9.1	233	26	52.7	8.95	0.52	7.0	264	40	2.1	-30	-14
EST	11.3	1.35	0.12	9.8	236	24	52.6	8.41	0.50	7.4	293	39	2.4	-57	-15
LBE	7.7	0.89	0.12	10.1	171	17	51.5	8.27	0.47	7.4	295	42	2.8	-124	-26
LBR	9.2	0.99	0.11	10.8	210	19	46.1	6.80	0.41	7.9	364	46	2.8	-154	-27
MAR	6.0	0.35	0.10	19.8	161	8	49.2	9.35	0.53	6.2	260	37	13.6	-99	-29
QUG	5.9	0.82	0.12	8.3	121	15	43.6	8.57	0.52	6.1	219	38	2.2	-98	-23
UPE	10.6	1.22	0.14	10.1	197	19	55.8	8.37	0.45	7.8			2.4		

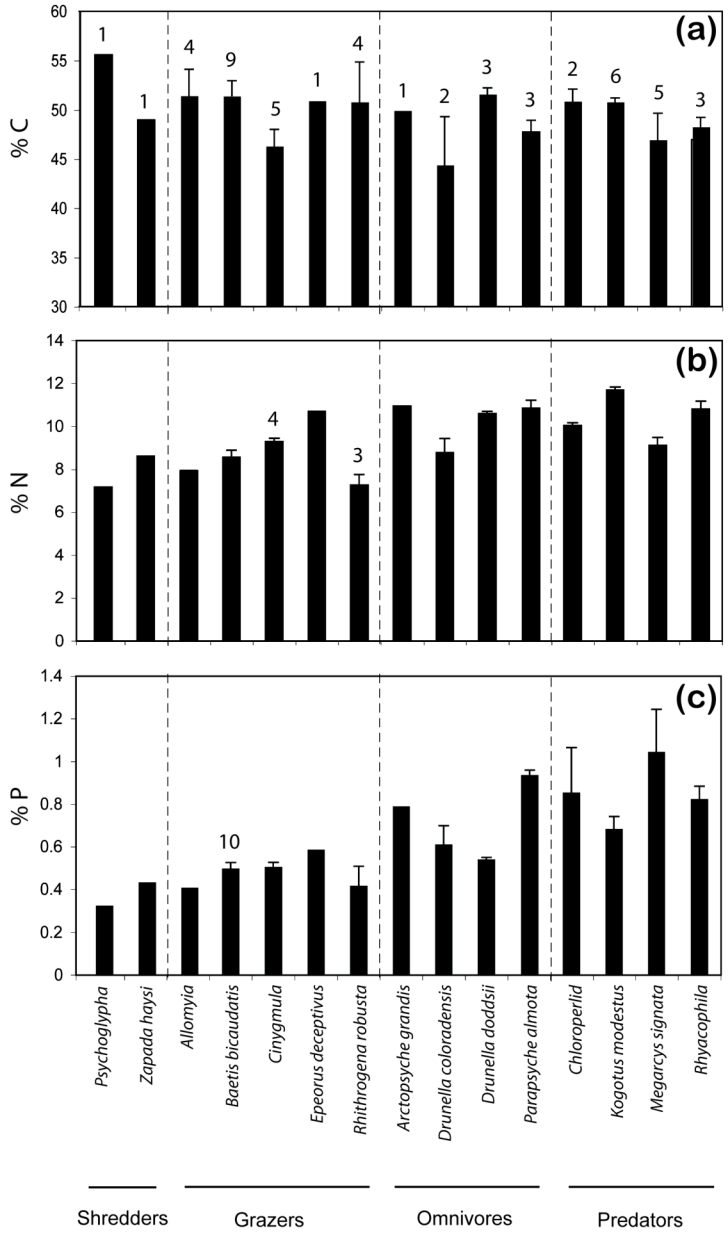

Figure 2.1. Means ± 1 SE for carbon (a), nitrogen (b), and phosphorus (c) content of benthic invertebrates ordered by functional feeding group. The number above each bar in the top panel indicates the number of streams represented. Numbers above bars in panels (b) and (c) indicate numbers of represented streams that differ from those indicated in panel (a).

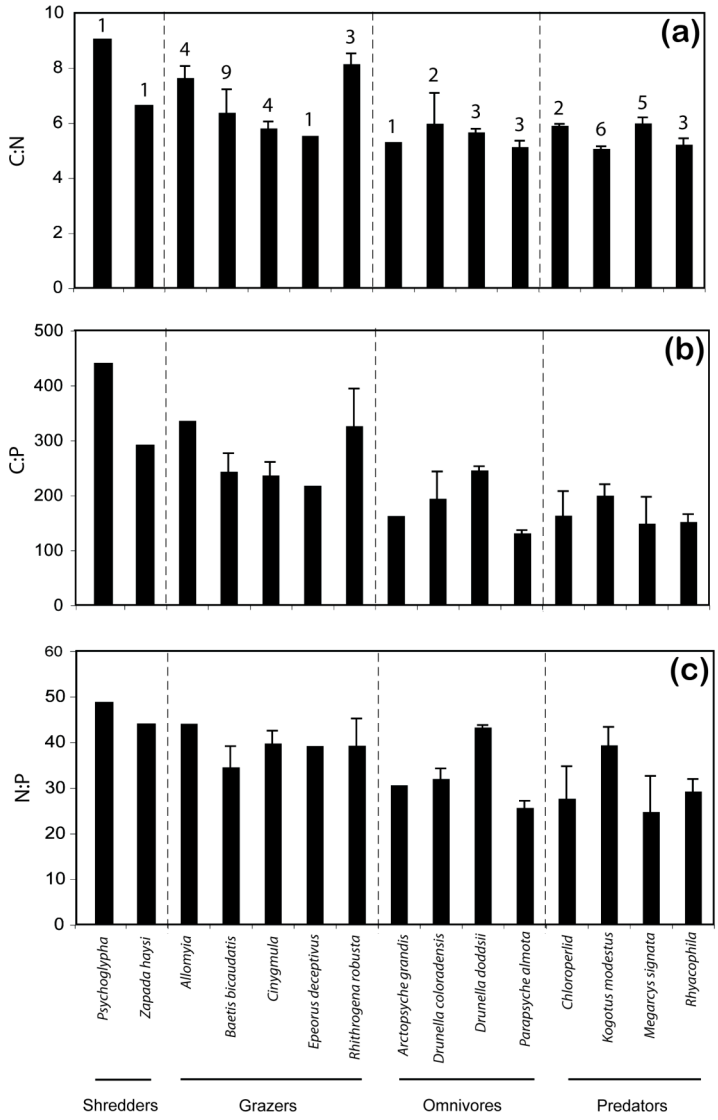

Figure 2.2. Means ± 1 SE for C:N (a), C:P (b), and N:P (c) ratios of benthic invertebrates ordered by functional feeding group. The number above each bar in the top panel indicates the number of streams represented.

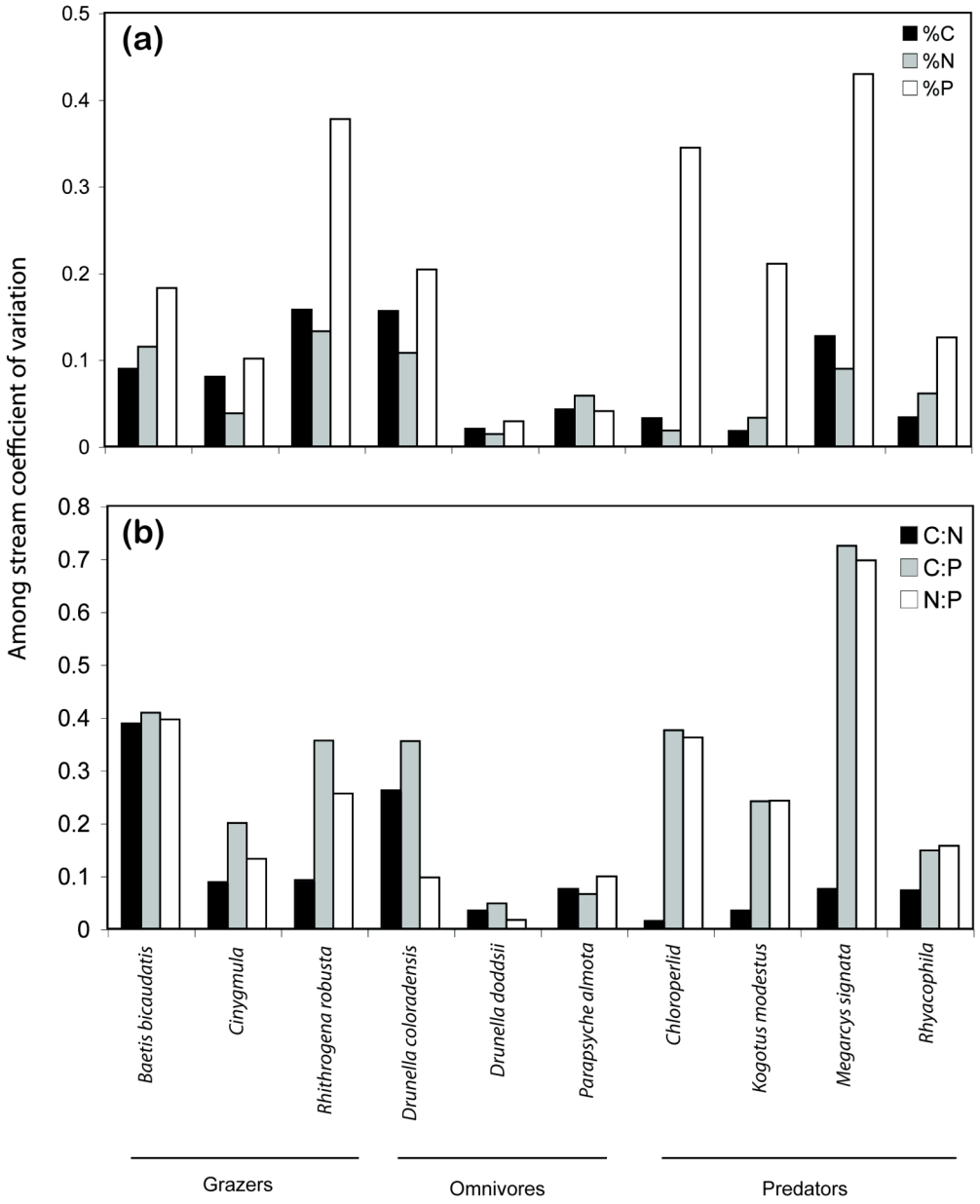

Figure 2.3. Among-stream coefficient of variation (CV) for %C, %N, %P (a) and C:N, C:P, and N:P (b) for benthic invertebrates found in at least three sub-alpine streams.

followed by omnivores, grazers, and then shredders. Correspondingly, C:N, C:P, and N:P were lowest for predators, followed by omnivores, grazers, and then shredders (Table 2.4).

Consumer-resource elemental imbalances

Arithmetic C:N, C:P, and N:P imbalances between epilithon and invertebrate grazers (mean of all grazer taxa present in each stream) varied moderately among streams (range C:N imbalance: 2.1 to 13.6, range C:P imbalance: -30 to -154, range N:P imbalance: -14 to -29; Table 2.3). Regressions between epilithon and grazer nutrient content were not significant (see Appendix 2A, Figure 2A). Invertebrate C:N ratios were lower than C:N ratios for epilithon across all streams, whereas invertebrate C:P ratios were greater than epilithon C:P ratios for all streams, suggesting possible N limitation of grazers. This was in contrast to limitation of algal communities, which were typically P driven (Table 2.2). A scatterplot of log C:N versus log C:P for all samples grouped by FFG reveals that epilithon generally had lower C:P but greater C:N ratios compared to grazers, although there was relatively more overlap for C:P ratios (Figure 2.4). Predator C:P was typically lower than that of grazers but overlapped with epilithon C:P ratios.

Tropho-chemical food webs

Stoichiometric (2007) and biomass (2006) data were combined to create a tropho-chemical food web diagram (*sensu* Sterner et al. 1996, Figure 2.5), a representation of C, N, and P stoichiometric patterns at the community level. The trophochemical food web depicts the nutrient pools contained in each taxon per square meter. The FFG effect was significant for C ($F_{2,28} = 7.2$, $P = 0.012$), N ($F_{2,28} = 7.2$, $P = 0.008$), and P ($F_{2,28} = 7.5$, $P = 0.007$) pools. Grazers contained larger amounts of C per

Table 2.4. Results of mixed effects ANOVA models and the mean ± 1 SE of carbon I, nitrogen (N), and phosphorus (P) content and C:N, C:P, and N:P ratios for invertebrate functional feeding groups.

FFG or statistic	%C	%N	%P	C:N	C:P	N:P
Shredder	52.3 ± 3.3	7.9 ± 0.7[a]	0.38 ± 0.05[a,b]	7.8 ± 1.2[a]	365 ± 75[a]	46 ± 2[a]
Grazer	50.2 ± 1.0	8.5 ± 0.2[a]	0.49 ± 0.02[a]	7.0 ± 0.2[a]	276 ± 15[a]	40 ± 2[a]
Omnivore	48.9 ± 2.0	10.1 ± 0.4[b]	0.61 ± 0.05[b,c]	5.7 ± 0.3[b]	214 ± 20[b]	38 ± 3[a]
Predator	48.9 ± 0.8	10.6 ± 0.3[b]	0.86 ± 0.06[c]	5.4 ± 0.1[b]	164 ± 15[b]	31 ± 3[a]
F – value	0.54	11.0	14.6	10.9	11.0	3.2
P – value	0.66	< 0.001	< 0.001	< 0.0001	0.001	0.03

square meter of streambed than predators and epilithon (grazers > epilithon > predators), and more N and P than epilithon (grazers > predators > epilithon).

Predictors of stoichiometric patterns

Several stream characteristics were significant predictors of stoichiometric patterns in benthic resources and consumers (Table 2.5). Most significant predictors described physical characteristics of streams (Shields' stress, Pfankuch's index score, maximum discharge, number of hours 80-100% bankfull flow from 2005-2008, maximum temperature, substrate mean particle size, and stream width). The only significant biological parameters were epilithon C:P (a predictor of grazer C:N) and mayfly density (a predictor of epilithon C:P and N:P). Furthermore, there were no significant predictors related to dissolved nutrient availability (i.e. specific conductivity, dissolved inorganic N, soluble reactive P, P limitation index). Shields' stress was the only significant predictor of epilithon C:N ($r^2_{adj} = 0.59$; Table 2.5). Mayfly density was the best single predictor of epilithon C:P ($r^2_{adj} = 0.58$; Table 2.5), and maximum discharge and stream width were the best single predictors of epilithon N:P ($r^2_{adj} = 0.68$ and 0.65, respectively; Table 2.5). Both parameters were collinear with each other ($r^2 = 0.95$, $P < 0.0001$) and produced similar levels of significance ($P = 0.002$ and 0.003, respectively; Table 2.5). The single best predictors of grazer nutrient content were epilithon C:P for grazer C:N ($r^2_{adj} = 0.42$; Table 2.5). No predictors were significant for grazer C:P. When multiple regression models were significant for predicting response variables (i.e. epilithon C:P and N:P, grazer C:N) they were more powerful than simple linear regression models. Pfankuch score and maximum discharge best explained epilithon C:P variability ($r^2_{adj} = 0.83$; Table 2.5), and Shields' stress and maximum discharge best explained epilithon N:P variability ($r^2_{adj} = 0.87$; Table 2.5). Grazer C:N was best explained by the total number of hours

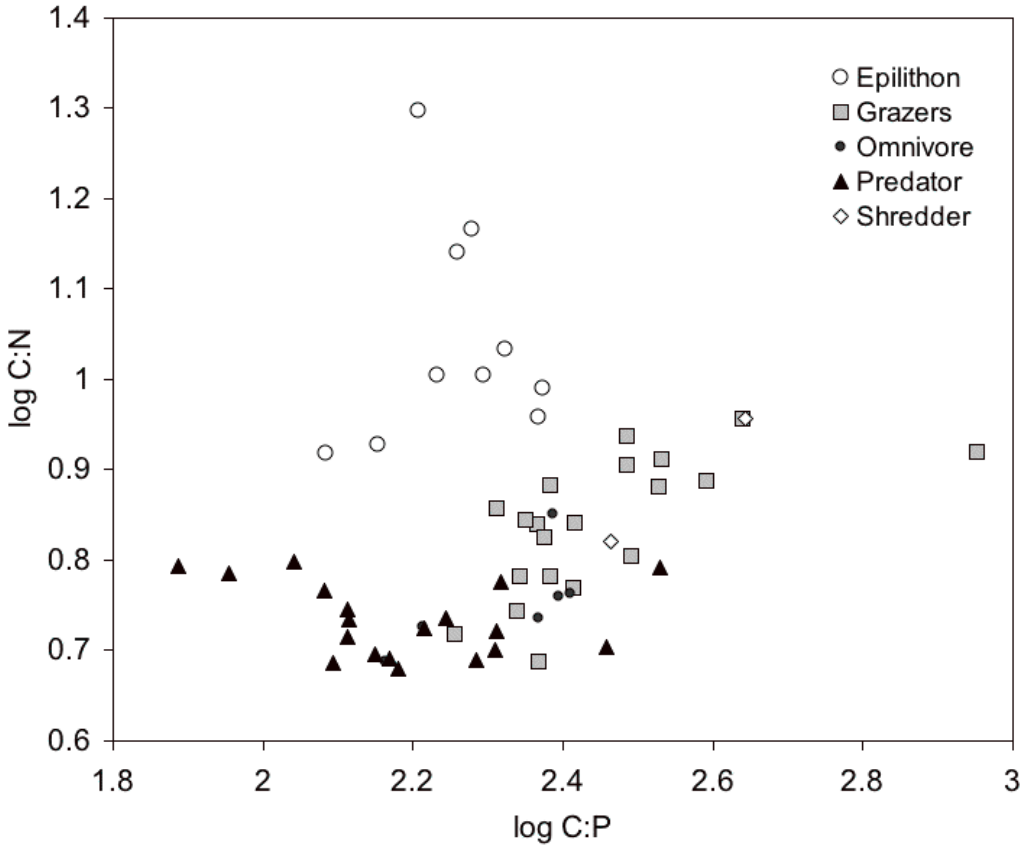

Figure 2.4. Scatterplot of C:N and C:P ratios of epilithon and invertebrate functional feeding groups (FFG) across all streams. Points represent individual taxa within FFGs. Note log transformation of axes.

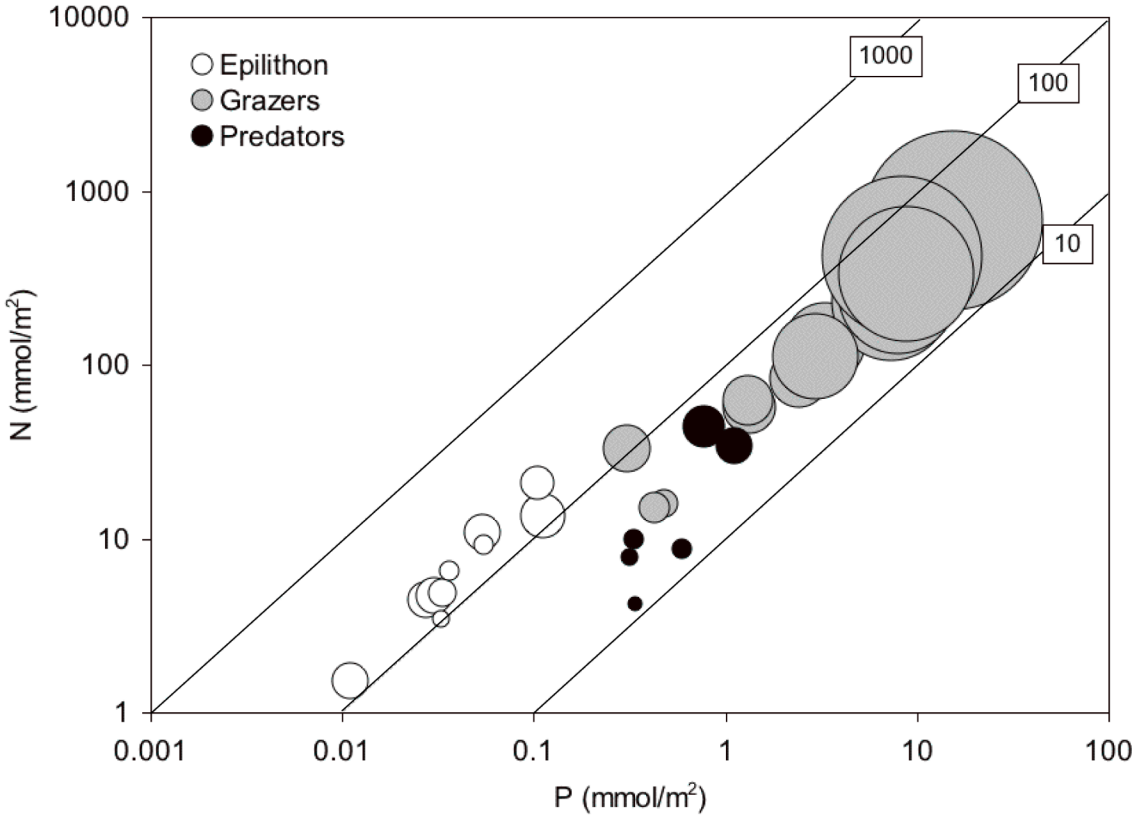

Figure 2.5. Tropho-chemical diagram showing nutrient pools (expressed as mmol/m^2) contained within benthic taxa. Circles represent a taxon within a stream and circle size indicates relative size of carbon pool for a taxon. Lines represent molar N:P ratios indicated in boxes. Note log scale.

Table 2.5. Statistically significant ($P < 0.05$) simple linear regression (SLR) and multiple linear regression (MLR) models for epilithon and grazer elemental ratios. No SLR or MLR models were significant for grazer C:P or N:P.

Elemental ratio and regression	Regression equation	d.f.	r^2_{adj}	F	P	β_{std}
Epilithon C:N						
SLR	$y = 7.6 + 21.8x_1$	1,9	0.59	13.7	0.006	0.79
Epilithon C:P						
SLR	$y = 124.5 + 1.1x_7$	1,9	0.4	6.9	0.03	0.68
SLR	$y = 166.4 + 13.5x_3$	1.9	0.46	8.6	0.019	0.72
SLR	$y = 146.1 + 0.06x_4$	1,9	0.58	13.6	0.006	0.79
MLR	$y = 264.3 – 1.1x_2 + 16.2x_3$	2,9	0.83	22.8	0.001	-0.61, 0.87
Epilithon N:P						
SLR	$y = 0.4 + 1.5x_6$	1,9	0.40	6.9	0.03	0.68
SLR	$y = 12.1 + 0.01x_4$	1,9	0.48	9.4	0.015	0.74
SLR	$y = 7.8 + 0.2x_7$	1,9	0.5	9.8	0.014	0.74
SLR	$y = 22.9 – 32.0x_1$	1,9	0.59	13.7	0.006	-0.8
SLR	$y = 13.0 + 1.2x_8$	1,9	0.65	17.7	0.003	0.83
SLR	$y = 14.1 + 2.3x_3$	1,9	0.68	20.2	0.002	0.85
MLR	$y = 18.6 – 19.9x_1 + 1.6x_3$	2,9	0.87	30.3	<0.001	-0.49, 0.6
Grazer C:N						
SLR	$y = 4.9 + 0.18x_6$	1,9	0.33	5.5	0.047	0.64
SLR	$y = 4.6 + 0.01x_9$	1,9	0.42	7.4	0.026	0.69
MLR	$y = 4.3 + 0.001x_5 + 0.2x_6$	2,9	0.73	12.9	0.005	0.62, 0.67

x_1 = Sheilds' stress D50
x_2 = Pfankuch Score
x_3 = Maximum discharge (m^3/s)
x_4 = Mayfly density ($indiv/m^2$)
x_5 = Total number of hours 80-100% bankfull flow 2005-2008
x_6 = Maximum temperature
x_7 = Substrate median particle size
x_8 = Stream width (m)
x_9 = Epilithon C:P

at 80-100% bankfull flow between 2005 and 2008 and maximum temperature (r^2_{adj} = 0.73; Table 2.5).

DISCUSSION

Interestingly, epilithon in streams within the East River drainage basin had high nutrient content relative to many other lotic systems (Table 2.6), despite low dissolved inorganic P availability (Table 2.1). For example, Cross et al. (2003) and Bowman et al. (2005) reported epilithon C:P values of 1741 and 3900 respectively, compared to an across-stream average C:P of 184 in this study. Epilithon is a complex amalgam of living and dead organic matter including algae, heterotrophic bacteria, metabolic byproducts, and detritus; as such, there are many pathways by which epilithon stoichiometry may differ among systems. A meta-analysis of over 5000 studies suggests that algal C makes up only a small proportion (average = 8.4%) of epilithon biomass (Frost et al. 2005). However, many alpine and sub-alpine watersheds likely have considerably less detritus than aquatic systems located at lower elevations, due to shorter growing seasons and reduced sources of allochthonous detritus. In addition, short growing seasons and frequent disturbance events typical of high-elevation streams create conditions that may favor relatively fast-growing, and therefore P-rich (growth rate hypothesis, Sterner and Elser 2002), taxa. Harsh abiotic conditions may "lock" algae in an early successional stage of rapid growth, precluding accumulation of senescent algae with high C:nutrient ratios and selecting for organisms with faster growth rates (Sterner and Elser 2002). This view corresponds to our expectation following the growth rate hypothesis in Sterner and Elser (2002) that conditions favorable to organisms with fast growth rates would have high P demand and low tissue C:P. High algae:detritus ratios, prevalence of fast-growing taxa, and

Table 2.6. Comparison of carbon I, nitrogen (N), phosphorus (P), and C:N, C:P, and N:P ratios of lotic organisms. Means or ranges are included when data were available. Only data from reference streams are included.

Reference	Location	Taxon	%C	%N	%P	C:N	C:P	N:P
This study	Colorado, USA	Epilithon	9.5	1.1	0.13	12	184	17
		Baetis bicaudatis	51	8.6	0.52	7.1	270	38
		Cinygmulid mayflies	46	9.3	0.51	5.8	237	40
		Grazers (all species)	50	8.5	0.49	7	276	40
Cross et al. (2003)	North Carolina, USA	Epilithon	16.5	2.2	0.02	8.7	1741	201
		Scraper-herbivores				6.2	369	59
Bowman et al. (2005)	Alberta and British Columbia, Canada	Epilithon	13	0.6	0.1	66	3900	120
		Heptageniid mayflies	42	9.2	0.5	5.0	210	40
Evans-White et al. (2005)	Wisconsin and Indiana, USA	Epilithon				11-15*	200-400*	15-35*
		Benthic insects				4.5-8.5*	150-750*	25-135*
Fink & Von Elert (2006)	Lake Constance, central Europe	Epilithon				30-45*	350-1200*	
		Radix ovata				5*	175-225*	
Liess & Hillebrand (2005)	Lake Erken, Sweden	Epilithon				9.57-13.53	247-435	23.6-44.9
		Benthic invertebrates				5.00-6.04	168-208	29.5-39.7
Singer & Battin (2007)	Austria	Epilithon	5.9	0.8	0.15	7.9	50.5	
		Baetis	45.6	10.1	1.07			
		Rhithrogena	48.3	9.8	0.94			
Rothlisberger et al. (2008)	Idaho, USA	Epilithon		1.82	0.40			
		Cinygmulid mayflies	46.78	10.05	1.00			

* indicates approximate values inferred from published graphs

scarcity of senescent organic matter may all influence the stoichiometry of epilithon in streams of the East River drainage basin, with the overall outcome of increased N and P content and low C:nutrient ratios. Furthermore, algal biomass was generally low (1.4 – 5.9 mg/m^2 chlorophyll a; J. Moslemi, *unpublished data*), suggesting low areal uptake rates of dissolved inorganic nutrients by algal communities. Low demand for dissolved nutrient resources due to low algal biomass may have permitted nutrient-rich, fast-growing species (e.g. bacteria) to outcompete other epilithon taxa, despite low dissolved P concentrations and widespread P limitation of algal growth (Table 2.2).

Nutrient content of benthic invertebrates were similar to those found in a study of Canadian Rocky Mountain streams (Bowman et al. 2005; Table 2.6). Elemental composition of individual invertebrate taxa did not differ across streams included in this study, consistent with our hypothesis that consumer elemental composition would be homeostatic across the stream network. Furthermore, when invertebrate taxa were pooled into FFGs (i.e. grazers, omnivores, and predators; all arthropods) and analyzed separately, each group had similar elemental composition across the watershed despite differences in community composition and physical-chemical profiles of streams. Within FFGs, omnivores exhibited the most differences in elemental composition among taxa, likely due to the larger breadth of food resources and nutrient compositions available for exploitation by this group. Across FFGs, differences were significant for all nutrient content descriptors except % C (Table 2.4). N and P contents were higher and C:P, C:N, and N:P ratios were lower for predators, which is generally consistent with results from other studies (Fagan et al. 2002, Cross et al. 2003). Lower C:nutrient composition of prey consumed by predators has been put forth as a possible explanation for this pattern (Fagan et al. 2002). However, results from this study and previous studies are not entirely

consistent; Cross et al. (2003) observed that FFGs contained different % C values in a detritus-based system, and Evans-White et al. (2005) found higher % N but not % P in predators in streams within watersheds that contained primarily deciduous forest and agriculture. These discrepancies may be due to differences in composition of basal resources and consumer communities across systems.

Consumer-resource elemental imbalances were estimated using arithmetic differences between C:N and C:P ratios of epilithon and invertebrate grazers (Table 2.3). Using this estimate, grazers were out of balance with C:N ratios of their food consistently in all 10 study streams, suggesting that grazers may be slightly limited by N in this system. The degree of C:N imbalance was variable among streams, ranging from 2.1 to 13.6 (Table 2.3). Interestingly, grazers had higher C:P ratios relative to epilithon, suggesting that P content is proportionately greater in basal food resources than in grazer body tissue. Observations of higher P content in aquatic herbivores relative to their food is not unprecedented. A meta-analysis of P limitation for the crustacean zooplankter *Daphnia* in lakes (Elser et al. 2000) found that ~22% of lake seston C:P ratios were lower than the corresponding requirements of *Daphnia* (Boersma and Elser 2006). In the few benthos studies in field settings for which data for stoichiometry of both primary consumers and basal resources are available, two (including this study) out of eight (25%) conducted in streams and lakes reported higher mean C:P values for primary consumers relative to epilithon (Table 2.6). Grazer N:P ratios were also consistently higher than those for epilithon, due to the high P content of basal food resources in study streams.

Pools of C, N, and P within benthic food webs were concentrated in the primary consumer trophic level (Figure 2.5). The much larger biomass of grazing invertebrates relative to epilithon or predatory invertebrates suggests that there may have been a strong top-down effect controlling the quantity of epilithon. This finding

corresponds with previous work in the East River drainage basin in which grazing invertebrates were experimentally removed, resulting in a 57% increase in algal biomass (Taylor et al. 2002). Overall, it appears that grazers in this high-elevation system are more likely to be limited by food quantity than food quality, as epilithon had high nutrient content and low biomass relative to grazers.

Theoretical and empirical exploration of conditions that lead to limitation by food quantity versus food quality suggest that ecosystems with low food quantity would not be expected to show food quality constraints on consumers (Hessen 1992, Sterner 1997). This is suggestion is based on the notion that under conditions of low food quantity, metabolic processes (which require large amounts of C) will be more important than production processes (which require relatively large amounts of N and P). The implications are that below a certain threshold of food quantity required for maintenance, the N and P content of food resources becomes increasingly irrelevant, as animals will mainly need C for metabolism. According to these models, food quality does not likely play a role in the consumer dynamics in our study system. However, Boersma and Kreutzer (2002) demonstrated that food quality consistently influenced growth rates of the zooplankter *Daphnia magna* at various levels of algal biomass, even when food quantity was held to very low levels. It may well be that ecological stoichiometry should be considered in systems where food quantity is low, although this issue remains to be resolved.

It is worth noting that quantification of elemental imbalances involving benthic epilithon and primary consumers is challenging due to the variable composition of epilithon as described above. The degree of selectivity with which grazers forage within epilithon mats is largely unknown, and further investigation is needed to accurately assess elemental mismatches between grazers and their ingested food resources. In addition, simple arithmetic differences between nutrient ratios of

consumers and their resources do not incorporate animal bioenergetics (e.g. differential assimilation of C versus P and respiration of ingested C) and may overestimate imbalances (Frost et al. 2006). Therefore, the imbalance observed between the C:N of grazers and their resources represents a maximum estimate. Since we found little convincing evidence for food quality constraints for grazers in this system, we did not include additional metrics of imbalance.

Variability in epilithon nutrient content at the regional scale was best described by physical attributes of streams (Table 2.5). Characterizations of disturbance regime (e.g. Shields' stress, Pfankuch's index score, and total number of hours at 80-100% bankfull flow over a 3-year period), stream size (e.g. maximum discharge, stream width, median particle size), and maximum temperature were all significant predictors of epilithon C, N, and P stoichiometry. Density of mayfly grazers, a prevalent phylogenetic group that includes taxa with high biomass-specific impact on primary producers (Alvarez and Peckarsky 2005), was also significantly correlated with epilithon C:P and N:P ratios and was the only biological variable that predicted epilithon nutrient composition. Previous empirical studies have found that grazing by invertebrates can impact epilithon nutrient content (Hillebrand and Kahlert 2001, Liess and Hillebrand 2006). A meta-analysis of 119 epilithon-grazer experiments indicated that grazers generally increase N and P content of epilithon across systems, although the direction and magnitude of effects depended on grazer stoichiometry, and C:P ratios were positively correlated with grazer biomass (Hillebrand et al. 2008). The results of our study were partially consistent with these findings, as mayfly density was positively correlated to epilithon C:P and N:P ratios, an outcome further supported by microcosm experiments (see Chapter 3). Contrary to our expectations, no chemical variables characterizing dissolved nutrient availability or degree of nutrient limitation correlated with epilithon nutrient content. Previous

studies have demonstrated that epilithon nutrient content increases with dissolved nutrient availability (Stelzer and Lamberti 2002, Bowman et al. 2005, Singer and Battin 2007); however, natural variability in dissolved inorganic N and P at the watershed scale may not be large enough to influence epilithon stoichiometry. Although biological and chemical variables have been demonstrated to impact nutrient content of primary producers, physical properties such as ecosystem size and disturbance regimes may play a relatively larger role in shaping producer stoichiometric templates in some natural systems with low variability in dissolved nutrient concentrations. Variables may have opposing or interacting effects on basal resource stoichiometry that cannot be disentangled if predictors are assessed in isolation or in small combinations.

In describing patterns in consumer-resource stoichiometry in high-elevation streams, we found a decoupling of growth-limiting factors at different trophic levels: while algal growth is generally limited by P availability, our results suggest that P content of food resources did not limit growth of primary consumers, which were instead likely limited by C. Our results suggest that food quantity is a more important driver on consumer dynamics than food quality in these systems. In addition, our analysis identified environmental characteristics that influence stoichiometry of basal resources in these ecosystems. We have shown that physical variables are most strongly associated with epilithon nutrient content, possibly due to low variability in chemical and biological characteristics among streams at the watershed scale. Further analysis the factors that shape stoichiometric constraints within diverse ecosystems will improve predictions of the consequences of environmental change on food web dynamics.

APPENDIX 2A

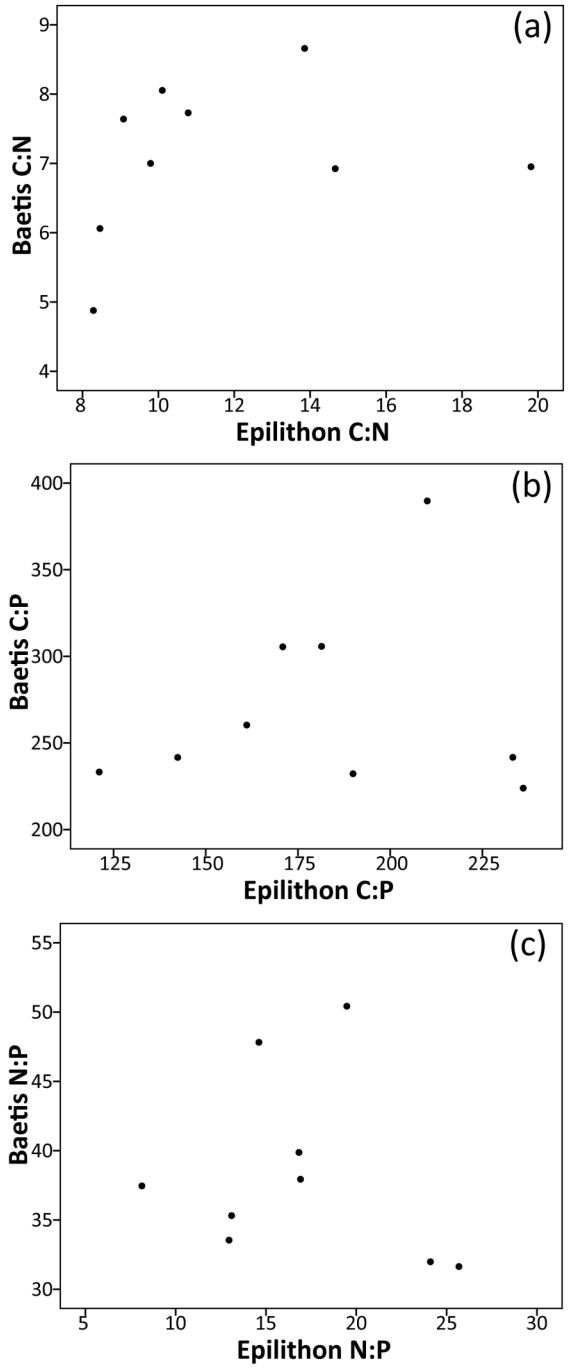

Figure 2A. Scatterplots of epilithon and a dominant grazer taxon (*Baetis bicaudatis*) C:N (a), C:P (b), and N:P (c) ratios. No linear regressions were significant (P = 0.48, 0.73, and 0.62, respectively).

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CHAPTER 3

CONSEQUENCES OF TOP-DOWN AND BOTTOM-UP FORCES ON CONSUMER-RESOURCE STOICHIOMETRY AND ELEMENTAL IMBALANCE ACROSS EXPERIMENTAL SCALES

ABSTRACT

Nutrients and consumers can constrain carbon I, nitrogen (N), and phosphorus (P) content of autotrophs, which has significant implications for trophic interactions. Consumer-resource interactions are influenced by mismatches in the elemental composition of resource supply versus the nutrient demand of consumers. Since most studies of bottom-up and top-down controls on autotroph C:N:P have been conducted in simple consumer-resource systems, little is known about the influence of additional trophic levels on food web stoichiometry. We tested the effects of nutrients (N and P), grazers (stream insect larvae), and predator cues (trout odor) on consumer-resource C, N, and P stoichiometry. Our objectives were to: (1) test the effects of nutrients and consumers on C, N, P content of epilithon in high-elevation streams, (2) determine whether top predators modified the effects of nutrients and consumers on stoichiometric imbalance between resources supplied by autotrophs and the nutrient demand of consumers, and (3) determine whether nutrients had consistent effects across three experimental scales ranging from microcosms (~175 cm^2) to whole ecosystems. Added nutrients (N and P) increased epilithon P content across all scales, and increased epilithon N content only when whole-ecosystems were enriched. In comparison, grazers consistently increased C and N content, but not P content, of epilithon. Fish odor appeared to reduce the influence of grazers on epilithon C and N content in microcosms, but there were no corresponding effects of fish in mesocosm (600 cm^2) experiments conducted across fish and fishless streams. Our findings show that bottom-up and top-down forces can have different influences on autotroph C:N:P stoichiometry, and may therefore impose distinct constraints on elemental mismatches between consumers and their resources. The lack of congruency of nutrient and predator effects across scales supports the notion that multi-scale studies are necessary

to understand links between mechanism and consequences of top-down and bottom-up forces at different levels of ecological complexity.

INTRODUCTION

Widespread anthropogenic changes to global nutrient cycles and consumer-resource dynamics precipitate the need to predict consequences of these modifications in a variety of ecosystems. Since the time of the industrial revolution, human activity has doubled global pools of biologically active nitrogen (N) and phosphorus (P; Galloway et al. 1995, Vitousek et al. 1997, Filippelli 2008), changing the relative availability of elements that limit primary production in many ecosystems (Elser et al. 2007). Such alterations to N and P pools can release "bottom-up" constraints, causing shifts in species composition and energy pathways through food webs (Smith et al. 1999). Changes in the strength of "top-down" forces via heterotrophic consumption can likewise influence composition and dynamics of lower trophic levels (Power 1990, Worm et al. 2006, Beyer et al. 2007). Humans are increasingly altering top-down controls through the introduction of novel consumers (Welsh and Scott 1995) and reduction and extirpation of native consumers (Estes and Palmisano 1974, Beyer et al. 2007), often with large consequences on primary producer communities and food web dynamics.

The relative importance of top-down and bottom-up forces in constraining ecological processes has been the subject of decades of research (Hairston et al. 1960, Oksanen et al. 1981, Carpenter et al. 1987, Hairston and Hairston 1993, Borer et al. 2006). Building on previous work assessing the relative strength of nutrients versus consumer controls on producers, recent efforts have pursued the promising approach of highlighting reciprocal feedbacks and quantifying interactions between bottom-up

and top-down forces (Lamberti 1996, Carpenter et al. 1998, Flecker et al. 2002). The impetus for this approach has been due in part to recognition of the interdependent relationship between consumers and nutrients; consumers themselves represent nutrient pools that influence fluxes of limiting nutrients via a number of direct and indirect mechanisms, and primary productivity can influence the intensity of heterotrophic consumption and recycling of dissolved nutrients by consumers (Oksanen 1981, Schindler et al. 1996, Vanni et al. 1997, Vanni 2002, Flecker et al. 2002). However, there exists a need to broaden our understanding of ecological responses to resource and consumer control by expanding beyond investigations of producer biomass and production to explore outcomes for other ecologically important variables such as tissue nutrient content (Gruner et al. 2008).

Ecological stoichiometry – the mass balance of chemical elements in ecological interactions – provides a lens through which to broaden understanding of resource and consumer controls on ecological dynamics (Sterner and Elser 2002). Using ecological stoichiometry as a theoretical framework, effects of bottom-up and top-down forces can be quantified in currencies of producer nutrient content and mismatches between the chemical composition of herbivores and their food resources. Elemental composition [most often carbon : nitrogen : phosphorus (C:N:P) ratios] of primary producer communities can vary widely in response to environmental conditions; in contrast, empirical data have shown that consumers are constrained to relatively homeostatic states (Elser et al. 2000, Sterner and Elser 2002). Stoichiometric imbalances can occur when the elemental composition of a food source does not meet the elemental requirements of a particular consumer. Nutrients that limit consumer growth and reproduction will be retained while nutrients consumed in excess will be excreted or egested as waste, which may come with a metabolic cost (Boersma and Elser 2006). Ecological stoichiometry theory states that a consumer in

relative stoichiometric balance with a given resource supply will have fewer food quality constraints on growth, reproduction, and assimilation efficiency of digested food, and thus gain a competitive advantage over other consumers within a functional group (Sterner and Elser 2002).

The conceptual framework of ecological stoichiometry has most often been tested in pelagic freshwater systems, although it has been applied in terrestrial (Fagan and Denno 2004) and benthic systems (Cross et al. 2003, Bowman et al. 2005, Evans-White et al. 2005, Hildebrand et al. 2008, Hladyz et al. 2009). Previous studies have concluded that nutrient content of food sources can limit consumer growth rates (Frost and Elser 2002b, Stelzer and Lamberti 2002) revealing widespread food quality constraints on consumer dynamics. In benthic systems, the chemical profiles of epilithon (an important basal resource) are plastic, and can be influenced by nutrients and presence of grazers. Several benthic studies have demonstrated that epilithon nutrient content can be positively related to dissolved inorganic nutrient availability (usually N and P; e.g. Rosemond et al. 1993, Stelzer and Lamberti 2001, Frost and Elser 2002a, Cross et al. 2003, Liess and Hillebrand 2006), due in part to the capability of producers for "luxury consumption" and storage of nutrients in excess of metabolic and structural requirements during times of relative plenty. Also, grazers can increase nutrient content of epilithon via consumption and nutrient excretion (e.g. Rosemond et al. 1993, Hillebrand and Kahlert 2001, Frost et al. 2002a, Evans-White and Lamberti 2006). Consumption could influence nutrient content of epilithon via two pathways: (1) metabolically active cells remaining in epilithon mats after grazing may have greater biomass-specific production rates and N and P contents due to increased per-capita nutrient availability and/or shift in composition to more nutrient rich taxa, and (2) bioturbation caused by grazers as they move though epilithon mats may dislodge loosely-attached senescent and inorganic matter (Steinman 1996).

Although previous studies of benthic food web stoichiometry have provided excellent examples of the effects of bottom-up and top-down forces on producer elemental composition, empirical studies that are large-scale (e.g. Cross et al 2003) and incorporate multi-trophic interactions will generate a more complete understanding of consumer-resource stoichiometry in natural systems (Gruner et al. 2008). Indeed, results from mesocosm-scale experiments cannot be presumed to extrapolate to larger scales and should be corroborated by large-scale approaches (Peckarsky et al. 1997, Carpenter 1998, Schindler 1998, Pace 2001). A broad understanding of the influences of these forces on elemental imbalance of resource supply and demand will facilitate the development of stoichiometrically explicit food web models (e.g. Andersen et al. 2004) and improve predictions of when grazer and nutrient manipulations may or may not yield non-additive effects in ecosystems by incorporating metrics of food quality.

Little is known about the role of predators as top-down forces that influence epilithon nutrient content. Predators may reduce biomass or alter movement of grazers, potentially modulating the intensity and spatial pattern of herbivory (Power 1990, Schmitz 1997, Persson 1999). In streams, predators can alter the periodicity and amount of prey movement (e.g. Cooper et al. 1990, Sih and Wooster 1994). For example, predator-avoidance behavior may reduce foraging time of primary consumers by restricting grazers to nocturnal use of habitat in which they are vulnerable to visually feeding trout (Cowan and Peckarsky 1994, McIntosh and Townsend 1995, McIntosh et al. 2004), and addition of predator chemical cues can influence patterns of algal heterogeneity in whole-stream manipulations (McIntosh et al. 2004). The presence of predators can also have effects on other aspects of prey microhabitat selection such as prey movement rates and patch residence time (Lima 1998). These predator-induced behavior modifications could modulate the effect of

primary consumers on the overall nutrient content of basal food resources by reducing consumption rates and bioturbation of epilithon mats by grazing invertebrates.

In this study, we investigated the effects of nutrients and primary consumers on grazer-epilithon stoichiometric relationships in high-elevation streams and determined whether cues from predators (trout) could modify the effect of grazers on epilithon elemental composition. Our objectives in this study were threefold. First, we tested the relative and interactive effects of top-down and bottom-up controls on C, N, and P stoichiometry of basal resources. Second, we determined whether cues from top predators modified the effects of nutrients and consumers on the stoichiometric imbalance of resource supply and demand. The presence of top predators was either simulated experimentally (fish odor) or tested across a stream network comprised of fish and fishless streams. We also considered whether the influence of fish odor differed among primary consumers that varied in their vulnerability to top predators. Third, we tested the influence of manipulated dissolved nutrient availability at different scales (microcosm, mesocosm, and whole-stream experiments) to assess whether nutrient effects observed at small scales were observed in manipulations of natural systems. This cross-scale approach provides insight into the scale-dependency of observed responses to manipulations of nutrients. Our findings add a new dimension to previous research on bottom-up and top-down controls on producers, and by extension, highlight potential implications for food web dynamics.

We tested three main hypotheses based on observations from previous studies. First, we hypothesized that both nutrients and grazers would increase the N and P content of epilithon, thereby reducing the elemental imbalance between primary consumers and their resources. Several studies have shown that nutrient enrichment can increase nutrient content of epilithon (e.g. Rosemond et al. 1993, Stelzer and Lamberti 2001, Cross et al. 2003, Liess and Hillebrand 2006), and a meta-analysis of

grazer impacts on nutrient content of basal resources by Hillebrand et al. (2008) found that grazers generally increased the N and P content of epilithon. Second, we hypothesized that predators would reduce the impact of grazers on epilithon stoichiometry by altering foraging behavior of grazers vulnerable to predation. Studies in streams have demonstrated that predator-avoidance behavior may restrict foraging time of grazers (Cowan and Peckarsky 1994, McIntosh and Townsend 1995), and McIntosh et al. (2004) observed differences in foraging behavior when grazers were experimentally exposed to odor of predatory fish. Third, we expected the effect of nutrients on consumer-resource stoichiometry to differ across experimental scales due to inherent differences in physical conditions that in turn influence trophic interactions and the distribution of resources. Peckarsky et al. (1997) synthesized observations from several studies in streams in which large-scale patterns of abundance and distribution of individuals deviated from expectations based on small-scale studies of individual behavior. We anticipated that deviations associated with behavior and trophic interactions would preclude direct extrapolation of observations across scales in our study.

METHODS

Recognizing that scaling in ecological study is often challenged by tradeoffs between experimental control and incorporation of natural complexity, we tested our hypotheses in three different experiments that varied in scale. First, we determined whether the effects of nutrients and grazers on consumer-resource stoichiometry were influenced by simulated predation risk (fish odor) in microcosms (~170 cm^2 area, "microcosm experiment"), where we could investigate the effects of fish odor on individual grazer taxa (*Baetis* mayflies or *Allomyia* caddisflies) that differ in their

vulnerability to predation. Second, we tested the effect of nutrients and natural densities of grazer communities in larger chambers that we refer to as the "mesocosms" for the purposes of this paper (~600 cm^2 area) using water from 10 streams across a network consisting of five fish and five fishless streams ("mesocosm experiment"). Grazer assemblages were placed in mesocosms in proportion to the natural densities found in each corresponding study stream. Finally, we added nutrients to 50-m long reaches of similar, adjacent, fishless streams ("whole-stream manipulation") to determine how nutrients influenced consumer-resource stoichiometry in natural systems and whether effects were consistent across experimental scales.

Study Area

All experiments were conducted in the East River drainage basin near the Rocky Mountain Biological Laboratory in western Colorado, USA (38°57'0" N, 106°58'59" W; elevation 2900 m). The watershed is largely dominated by subalpine spruce-fir-aspen forests, and riparian willows border many streams. Streams of the East River drainage basin are either snowmelt- or spring-fed, and their streambeds are characterized by stony substrates. Lotic systems in this area are generally oligotrophic (dissolved inorganic N: 54-168 μg/L, dissolved inorganic P: < 1-4 μg/L) and primary producers are predominantly P limited (see Chapter 2). Although bryophytes and filamentous algae are present in many streams, epilithic diatoms dominate primary producer communities. The invertebrate community is primarily composed of insect larvae. Among the most dominant taxa are Ephemeroptera (mayflies), Trichoptera (caddisflies), Plecoptera (stoneflies), and Diptera (true flies). Some streams contain populations of brook trout (*Salvelinus fontinalis*), whereas others are fishless due to natural barriers to fish dispersal.

Microcosm experiment

To test the effects of nutrients, grazers, and predators on consumer-resource stoichiometry, we added nutrients, natural densities of mayfly (*Baetis bicaudatis*) or caddisfly (*Allomyia gnathos*) larvae, and top predator chemical cues (fish odor) to microcosms stocked with epilithon-covered ceramic tiles. This approach enabled us to quantify the stoichiometric consequences of (1) dissolved nutrient availability, (2) presence of two separate grazer taxa that differed in their vulnerability to fish predation, (3) influences of predator cues on grazer behavior, and (4) interactions between these three factors.

Filtered water from a small, fishless tributary to the East River was gravity-fed into 60 circular flow-through microcosms (diameter = 15 cm; see Fig. 1 in Peckarsky and Cowan 1991). Microcosms were constructed from plexiglass and housed in an opaque white greenhouse. We placed six ceramic tiles (13.32 ± 0.04 cm^2, mean $\pm$ 1 SE) in each microcosm. To generate similar starting resource levels, tiles were suspended in the water column of the East River for 10 days using a raft for colonization by primary producers. Average current velocity within microcosm chambers was 8.02 ± 0.29 cm/s (mean $\pm$ 1 SE), and water temperature ranged from 14.1 to 16.2 °C across chambers. Current velocity was measured with a Nixon micro-propeller flow meter (Charlton Kings Industrial Estate, Cheltenham, Gloucestershire, UK). Water temperature was recorded using Onset HOBO Pendant temperature/light data loggers (Pocasset, MA, USA). The duration of the experiment was two weeks (June 26—July 10, 2007), throughout which microcosm chambers were cleaned daily to avoid clogging. Nutrients, grazer, and predator cue treatments were randomized across microcosms. Three treatment factors were crossed in a 3 x 2 x 2 factorial experimental design for a total of 12 treatment combinations: grazers (none, *Baetis*, or *Allomyia*), nutrients (ambient or N and P added), and predator cues (none or fish odor

added). There were five replicates for each treatment.

For predator cue and nutrient treatments, fishless stream water was gravity-fed into 110 L plastic bins containing either two brook trout (*Salvelinus fontinalis*) collected from the East River, nutrient-diffusing pellets, or a combination of both. Nutrient-diffusing pellets released NH_4 and PO_4 continuously [as $(NH_4)_2SO_4$ and $(NH_4)_3PO_4$; N:P ratio = 0.625:1; Continuous Release Bloom Booster, Scotts Miracle-Gro Company, Marysville, OH, USA]. Water from the bins was transported and dripped into flow-through microcosms using tygon tubing at a rate of 4.0 $\pm$ 0.2 ml/s (mean $\pm$ 1 SE). Water samples were regularly collected from microcosms and filtered (Pall-Gelman A/E filters) for water chemistry analysis; NH_4 was immediately analyzed using a Turner Designs Aquaflour (Sunnyvale, CA, USA) following methods in Taylor et al. (2007), NO_3 was analyzed using a Dionex ICS-2000 Ion Chromatography system, and soluble reactive phosphorus (SRP) was measured using a spectrophotometer (Shimadzu 1240; molybdate blue method, Murphy and Riley 1962). Water samples used for NO_3 and SRP analysis were frozen and transported to Cornell University for analysis. Fishless stream water with ambient nutrient levels was dripped at a similar rate into mesocosms that did not receive nutrient or predator cue treatments to control for any unanticipated drip effects. Nutrients were replenished as necessary to maintain enrichment levels (target enrichment = ~10X above ambient NH_4 and SRP) and trout were fed a mixed diet of stream invertebrates every two days from the East River. Mean total length ($\pm$ 1 SE) of the four trout used in the experiment was 156 mm ($\pm$ 4.5).

We added 12 *Baetis* and 12 *Allomyia* to each of 20 microcosms (240 total individuals for each taxon) to generate densities of ~700 larvae m^{-2}, which falls within the range of densities observed in natural streams in the region (B. L. Peckarsky and A. R. McIntosh, *unpublished data*). Larvae were collected from fishless tributaries of

the East River. The remaining 20 microcosms did not receive grazers. *Baetis* is a highly mobile grazer that has been shown to have a high biomass-specific impact on epilithon biomass (Alvarez and Peckarsky 2005). In comparison, *Allomyia* is a relatively sedentary caddisfly grazer protected—and hindered—by a stony case that it builds from fine gravel. However, the stony case also protects *Allomyia* from fish predators, creating a tradeoff between grazing efficiency and vulnerability to top predators.

At the end of the experiment, grazers and epilithon were collected for subsequent analysis of C, N, and P content. Epilithon was collected by scrubbing the entire surface area of three tiles with a plastic bristle brush, generating a slurry of organic and inorganic matter. The remaining three tiles were analyzed for algal biomass; these data will be presented elsewhere (B. L. Peckarsky, A. R. McIntosh, J. M. Moslemi, *unpublished data*). Slurries were settled over a 24 h period after which water was decanted and the remaining concentrated sample was dried in a drying oven at 60 °C and stored in a desiccator until processed for elemental composition analysis. Due to limited epilithon biomass accrual in cold, high-elevation streams, all replicates for each treatment needed to be pooled into a single integrated sample to obtain sufficient material for elemental composition analysis. Grazers were collected using forceps; all individual larvae within a given microcosm were pooled into one sample for a total of five replicates per treatment. Larvae were kept in vials with streamwater for several hours to allow for clearance of guts and then frozen until further processed.

For elemental composition analysis, epilithon and macroinvertebrate samples were dried to a constant weight for at least 24 h, then ground to a fine powder and homogenized using a mortar and pestle. Subsamples were weighed on a microbalance (Mettler Toledo MX5) to the nearest μg and analyzed for C and N content using a CHNS elemental analyzer (Elementar Vario EL III). For P content, subsamples were

weighed into acid-washed Pyrex tubes, ashed at 500 °C, digested in 1 N HCl, and analyzed on a Shimadzu UV 1240 spectrophotometer (molybdate blue method, Murphy and Riley 1962). Ground citrus leaves and spinach (US National Institute of Standards and Technology, US Department of Congress) were used as standards for P analysis and were analyzed in each set of samples. Percent recovery of P from standards was typically 97-100%. Total C, N, and P are presented as percentage of dry mass, and elemental ratios are molar.

Mesocosm experiment

To test the effects of bottom-up and top-down processes on basal resource stoichiometry across a stream network, we added grazer communities at natural densities and nutrients to flow-through mesocosms located adjacent to ten study streams in the East River drainage basin. For each study stream, a set of four mesocosms were constructed from plastic buckets (diameter = 30 cm, area of bottom excluding drain ~ 600 cm^2) and received the following treatments: (1) no nutrients and no grazers, (2) grazer assemblage added, (3) nutrients added, and (4) grazer assemblage plus nutrients added (Appendix 3A). Treatments were randomized across mesocosms. Filtered water from each study stream was gravity-fed to each corresponding set of four mesocosms for total of 40 mesocosms across all streams. We collected epilithon-covered rocks from each study stream, removed any invertebrates present, scrubbed rocks with a plastic bristle brush to generate similar starting resource levels, and placed 10 rocks to cover the bottom of each mesocosm (see Appendix 3A for additional information on experimental design). We added grazers to mesocosms in similar densities as those observed in each corresponding study stream. To obtain natural densities, invertebrates were sampled at three random locations within 50 m reaches using a custom-made box sampler (0.104 m^2 area) fitted

with a 360 μm mesh drift net and preserved in a 95% ethanol solution. In the laboratory, invertebrates were identified to the lowest possible taxon (genus or species).

For nutrient enrichment treatments, stream water was gravity-fed into a separate plastic bin containing nutrient-diffusing pellets (as used in the microcosm experiment, described above). Water from this bin was dripped through tygon tubing to corresponding mesocosms at a rate of 7.2 $\pm$ 0.3 ml/s (mean $\pm$ 1 SE) to maintain nutrient enrichment levels (target enrichment = ~10X ambient NH_4 and SRP). Stream water with ambient nutrient levels was dripped into non-enriched treatments at a similar rate to account for any unanticipated drip effects. Samples were collected regularly from each mesocosm for water chemistry analysis as described above for the microcosm experiment. The duration of the experiment was two weeks (July 12—July 27, 2007), during which mesocosms were cleaned daily to avoid clogging.

At the end of the experiment, epilithon and one grazer mayfly taxon that was found in all study streams (*Cinygmula* spp.) were collected for subsequent analysis of C, N, and P content. Epilithon was collected by scrubbing the entire surface area of half of the rocks within each mesocosm using a plastic bristle brush. The remaining rocks were analyzed for algal biomass; these data will be presented elsewhere (B. L. Peckarsky, A. R. McIntosh, J. M. Moslemi, *unpublished data*). Before elemental analysis, epilithon slurries were filtered through a 500 μm sieve to remove invertebrates. *Cinygmula* individuals were collected from mesocosms using forceps. Epilithon and *Cinygmula* were processed and analyzed for elemental composition in the same manner as for the microcosm experiment described above.

Whole-stream manipulation

To test the effects of bottom-up processes on the consumer-resource

stoichiometry of natural streams, we conducted a whole-stream nutrient addition experiment. Four adjacent streams (B1, B2, B3, and B4) were included in the experiment, which was conducted between July 1 and July 29, 2008. Within each stream, 50-m study reaches were delineated and a four-week continuous nutrient drip was set up at the top of the study reaches of two streams. In enriched streams, NH_4 (as NH_4Cl) and PO_4 (as KH_2PO_4) were simultaneously released at a constant rate using a peristaltic pump (Fluid Metering, Inc., Syosset, NY, USA) to increase NH_4-N and SRP to ~10X ambient concentration. Water samples for nutrient analyses were collected and filtered (Pall Gelman Type A/E filters) at three stations within in each study reach and at one station above the nutrient release site before the initiation of the nutrient release and at weekly intervals for the duration of the experiment. Concentrations of NH_4, NO_3, and SRP were analyzed in the same manner as for the previous experiments described in this study. Stream discharge was quantified using a Marsh-McBirney Flo-Mate meter (Frederick, Maryland, USA) following the velocity-area method in Gore (2006).

To assess the consequences of nutrient addition on biomass and nutrient content of basal resources, we collected samples of epilithon from rocks before (initial) and at the end (final) of the four-week nutrient addition. To assess epilithon biomass, epilithon-covered rocks were collected from five random transects within the study reach; we scrubbed the entire surface of rocks using a plastic bristle brush to generate a slurry of organic and inorganic matter (one rock per slurry, five slurries per stream). A subsample of known volume was removed from the slurry using a pipette and filtered onto pre-combusted glass fiber filters (Pall-Gelman, Type A/E) for analysis of chlorophyll *a* (as an indicator of algal biomass) and ash-free dry mass (AFDM; as an indicator of organic material biomass). Upon filtration, chlorophyll *a* samples were extracted in 90% buffered ethanol for 24 h and analyzed following

fluorometric methods in Arar and Collins (1997) using a Turner Designs Aquafluor (Sunnyvale, California, USA). We processed AFDM samples following the methods in Wallace et al. (2006). To generate areal estimates of algal biomass and AFDM, we traced rocks used for each slurry and calculated areas of tracings using a leaf area meter. At the same five transects within each stream, additional rocks were scrubbed to collect epilithon samples for C, N, and P content; epilithon was processed and analyzed as described above for the previous experiments.

In addition, we measured algal biomass on tiles at weekly intervals during and after the nutrient addition. We placed 12 ceramic tiles (56.25 cm^2 area) at five randomly chosen sites (60 total tiles) in the study reach of each stream. Tiles were placed in streams at the initiation of the nutrient drip; one tile from each site was collected every week during the nutrient drip and for an additional six weeks after the drip terminated (June 28 – September 4, 2008) in order to assess changes in algal biomass once nutrients returned to ambient levels. Upon collection, tiles were immediately submerged in 90% buffered ethanol for 24 h to extract chlorophyll *a*. Chlorophyll *a* was analyzed as described above for rocks. At the end of the enrichment period, qualitative samples of algae were collected from rocks and preserved using a Lugol's iodine solution for identification of major taxa.

Invertebrates were sampled at five random locations within each study reach using a custom-made box sampler (0.0729 m^2 area, 360 μm mesh size) and preserved in (95%) ethanol. The five most dominant taxa (*Baetis bicaudatis*, *Cinygmula* spp., *Zapada haysi*, *Neothrema* spp., and Chloroperlidae) were identified from previous sampling efforts (B. L. Peckarsky, *unpublished data*). Several individuals of each of these taxa were collected from streams using forceps and dip nets for analysis of body C, N, and P content. Larvae were kept in vials with stream water for several hours to allow for clearance of guts and then frozen until processed and analyzed in the same

manner as described previously for the microcosm experiment.

Statistical analyses

We compared nutrient composition (%C, %N, %P, C:N, C:P, N:P) of epilithon (mesocosm experiment) and grazers (microcosm experiment) among treatments using multivariate analysis of variance (MANOVA) to account for experiment-wise error associated with testing effects on interdependent response variables. Due to low epilithon biomass accrual on tiles in the microcosm experiment, all replicates for each treatment needed to be combined to obtain sufficient material for elemental analysis, and therefore no statistical analysis could be performed. We used Wilks' likelihood ratio test to determine MANOVA significance. Significant variables in MANOVA tests were subsequently analyzed with three-way ANOVAs followed by Tukey's HSD multiple comparison tests (PASW software, version 18.0). Data were log transformed as necessary to satisfy ANOVA assumptions of normality and equal variance.

In the whole-stream manipulation, we used repeated-measured ANOVAs (weekly intervals for algal biomass on ceramic tiles; before and after nutrient addition for all other parameters) to test the effect of enrichment on producer and consumer communities. We adjusted the significance criterion (α) using a standard Bonferroni correction based on the number of comparisons conducted within a related group of response variables [e.g. six elemental composition variables (% C, % N, % P, C:N, C:P, N:P) resulted in a corrected $\alpha = 0.05/6 = 0.008$] to control for experiment-wise error. Tests with P-values that fell within a Bonferroni-corrected α of 0.008 and a standard α of 0.05 were considered marginally significant. To depict effects of enrichment on community-level C, N, and P pools, stoichiometric and biomass data for epilithon and invertebrates were combined (nutrient content multiplied by biomass) to create trophochemical food web diagrams (*sensu* Sterner et al. 1996).

Trophochemical food webs depict pools of elements contained in each taxon per square meter of streambed, and were constructed for initial (before enrichment) and final (after four weeks of enrichment) time periods.

Elemental imbalances between grazers and producers were quantified in two ways. First, we calculated the arithmetic difference between consumer and producer C:N:P ratios (*sensu* Sterner and Elser 2002). Since this method overestimates imbalances by ignoring differences in nutrient assimilation efficiencies and C lost via respiration, we also used the threshold elemental ratio (TER) approach (Urabe and Wantanabe 1992, Sterner 1997, Frost et al. 2006). The TER approach combines bioenergetics with body nutrient content to assess nutrient deficiency in heterotrophs, indicating the point at which limitation of consumers switches from energy I to mineral nutrient. When consumers are below the TER they are not constrained by the nutrient content of their food. If the food elemental composition exceeds consumer TER, consumers must dispose of ingested C that is in excess relative to their somatic requirements in order to obtain sufficient quantities of limiting mineral nutrients. Frost et al. (2006) estimated the TER of C and P ($TER_{C:P}$), the threshold at which limitation shifts from assimilation of C to assimilation of P, for 41 aquatic consumers and found that the average $TER_{C:P}$ across taxa was 2.4 times greater than body C:P (see Appendix 3B for additional information on calculation of $TER_{C:P}$). We assumed a factor of 2.4 times body C:P content to estimate $TER_{C:P}$ of consumers in our assessment. Imbalance using this approach was calculated as the difference between consumer $TER_{C:P}$ and dietary (i.e. producer) C:P (Imbalance = $TER_{C:P} - Diet_{C:P}$). Positive values indicated C or energy limitation, whereas negative values indicated P limitation.

RESULTS

Microcosm experiment

Microcosms receiving nutrient enrichments were elevated to ~20X ambient NH_4 and ~30X ambient SRP concentrations (Appendix 3C). Epilithon P content—but not N content—increased substantially with addition of dissolved N and P. Epilithon % P increased and C:P and N:P decreased in all cases when nutrients were added, independent of grazer or fish odor treatment (Table 3.1). In addition, under conditions in which no nutrients or predator cues were added, the presence of grazers increased the C:P ratio of epilithon (Table 3.1). However, due to the low epilithon biomass present on tiles at the end of the experiment, all replicates for each treatment needed to be combined to obtain sufficient material for elemental analysis, and no statistical analysis could be performed.

MANOVA results indicated that elemental composition (% C, % N, % P, C:N, C:P, N:P) of grazers differed among taxa (Wilks' $\lambda = 0.435$, $P = 0.001$) and nutrient treatments (Wilks' $\lambda = 0.350$, $P < 0.001$), with a significant species x nutrients interaction (Wilks' $\lambda = 0.471$, $P = 0.003$). Individual ANOVAs showed that percent C did not differ systematically between grazer taxa ($P = 0.98$); however, variation in % N lead to significant differences in C:N among grazers, with *Baetis* having higher % N than *Allomyia* (Table 3.2, Figure 3.1). Percent C, N, P and C:P, N:P ratios of grazers differed among nutrient treatments by the end of the two-week nutrient addition, contrary to the assumption that grazer elemental composition would remain homeostatic over the duration of the experiment (Table 3.2, Figure 3.1). The addition of predator cues did not consistently influence elemental of composition of grazers (Wilks' $\lambda = 0.711$, $P = 0.184$). However, there was a significant taxon x predator cue interaction effect on grazer C:P, with predator cues generally increasing C:P of *Baetis*

Table 3.1. Summary of epilithon elemental composition in microcosms. "+NP" indicates treatments in which nutrients were enriched.

Treatment	Grazer	% C	%N	%P	C:N	C:P	N:P
Control							
	none	6.25	0.722	0.103	8.66	60.8	7.02
	Allomyia	6.62	0.754	0.099	8.79	66.9	7.61
	Baetis	7.70	0.888	0.095	8.67	81.0	9.35
Fish odor							
	none	6.12	0.706	0.103	8.47	59.4	6.85
	Allomyia	5.20	0.613	0.096	8.47	54.0	6.37
	Baetis	6.57	0.830	0.104	7.92	62.9	7.94
+NP							
	none	6.75	0.776	0.150	8.73	44.9	5.16
	Allomyia	6.98	0.801	0.140	8.73	50.0	5.74
	Baetis	6.03	0.786	0.132	7.68	45.7	5.95
Fish odor, +NP							
	none	6.94	0.754	0.182	8.78	38.2	4.15
	Allomyia	6.29	0.717	0.132	8.78	47.6	5.42
	Baetis	6.40	0.740	0.142	8.65	45.0	5.20

Table 3.2. Three-way ANOVA table for descriptors of grazer elemental composition in microcosms. Significant *P* values are given in bold.

Parameter	Effect	F	P
%C	Species (S)	0.001	0.979
	Nutrients (NP)	4.788	**0.037**
	Fish (F)	0.061	0.807
	S x NP	0.33	0.570
	NP x F	0.9	0.766
	S x F	0.984	0.329
	S x NP x F	1.436	0.240
%N	S	13.717	**0.001**
	NP	7.88	**0.009**
	F	0.525	0.475
	S x NP	2.449	0.128
	NP x F	0.027	0.869
	S x F	0.842	0.366
	S x NP x F	2.552	0.121
%P	S	3.042	0.092
	NP	14.585	**0.001**
	F	2.788	0.106
	S x NP	2.153	0.153
	NP x F	0.05	0.825
	S x F	6.558	**0.016**
	S x NP x F	0.099	0.755
C:N	S	15.392	**<0.001**
	NP	0.494	0.488
	F	0.163	0.689
	S x NP	4.443	**0.044**
	NP x F	0.104	0.749
	S x F	3.842	0.060
	S x NP x F	0.196	0.661
C:P	S	3.821	0.060
	NP	24.368	**<0.001**
	F	2.853	0.102
	S x NP	3.548	0.070
	NP x F	0.015	0.904
	S x F	8.209	**0.008**
	S x NP x F	0.048	0.829
N:P	S	1.367	0.252
	NP	49.001	**<0.001**
	F	6.179	**0.019**
	S x NP	0.076	0.785
	NP x F	0.024	0.879
	S x F	3.526	0.071
	S x NP x F	0.785	0.383

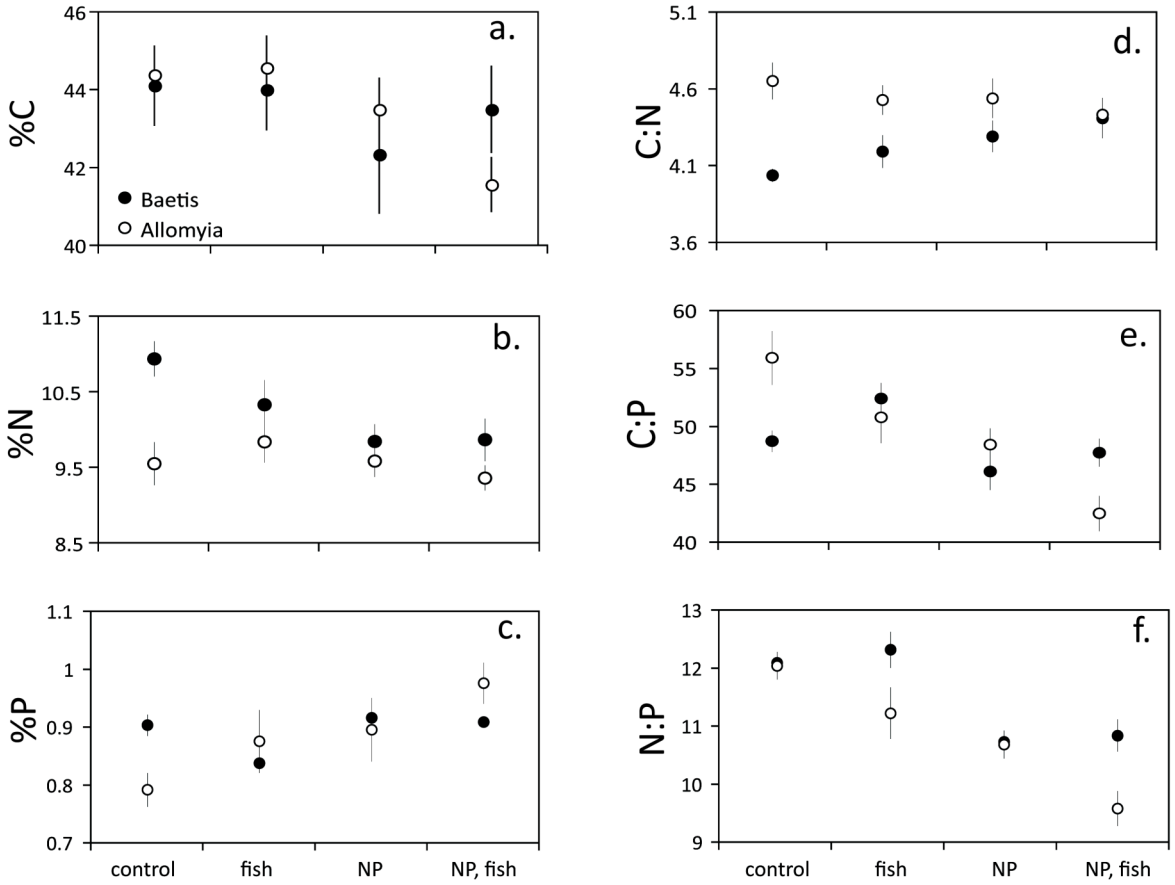

Figure 3.1. Mean (± 1 SE) % C (a), % N (b) % P (c), C:N (d), C:P (e), and N:P (f) of grazers in microcosms across nutrient enrichment and fish odor treatments.

while decreasing C:P of *Allomyia* (Table 3.2, Figure 3.1).

The range in epilithon C:P was greater than the C:N range across grazer, nutrient, and predator cue treatments (C:P range = 38.2 – 81.0, C:N range = 7.68 – 8.79; Table 3.1, Figure 3.2). Grazers and epilithon C:P ratios overlapped acrosstreatments, whereas there was no overlap in C:N ratios among the two trophic levels (Figure 3.2). Arithmetic C:P imbalances between epilithon and grazers among treatments were greater and showed more variability among treatments than C:N imbalances, driven largely by greater differences in epilithon P content (Figure 3.3; note differences in scales of the Y-axis). The addition of dissolved nutrients reduced C:P imbalances, but had no consistent influence on C:N imbalances (Figure 3.3). Differences among grazer species were relatively small for C:N imbalances compared to C:P imbalances, and *Baetis*-epilithon elemental imbalances exhibited the highest degree of change across treatments (Table 3.3, Figure 3.3). Imbalances calculated using the $TER_{C:P}$ method indicated that grazer taxa were limited by C or energy (as opposed to P) in all treatments (Table 3.3).

Mesocosm experiment

Mesocosms receiving nutrient enrichments were elevated to ~10X ambient NH_4 and SRP concentrations (Appendix 3C). At the end of the two-week experiment, epilithon elemental composition (% C, % N, % P, C:N, C:P, N:P) differed significantly in the presence of grazers (Wilks' $\lambda = 0.443$, $P = 0.001$), with the addition of dissolved nutrients (Wilks' $\lambda = 0.330$, $P < 0.001$), and between fish and fishless streams (Wilks' $\lambda = 0.504$, $P = 0.003$). There were no significant interaction effects among factors (grazers x nutrients: $P = 0.157$, grazers x fish: $P = 0.259$, nutrient x fish: $P = 0.584$, grazers x nutrients x fish: $P = 0.413$). Individual ANOVAs showed that grazers increased epilithon % C and % N, but had no effect on % P

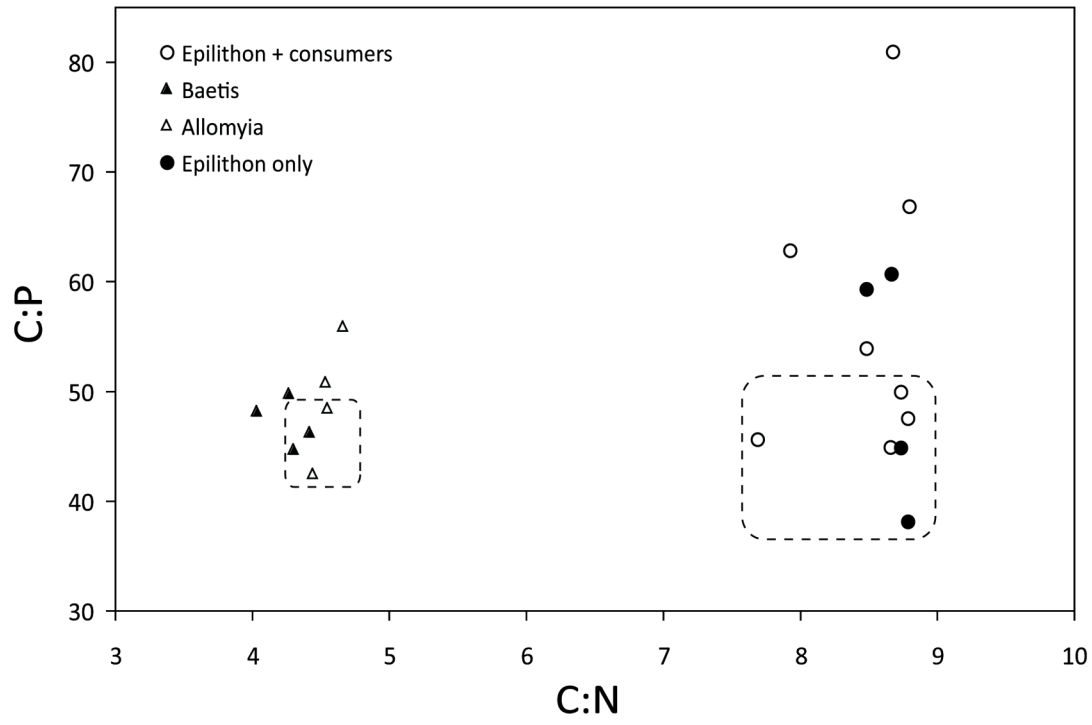

Figure 3.2. C:N:P ratios of epilithon and grazers in microcosms. "Epilithon + consumers" represents epilithon stoichiometry for treatments in which grazers were present, and "Epilithon only" represents epilithon in the absence of grazers. Treatments in which microcosms were enriched with nutrients are indicated by dashed rectangles. Note that enriched treatments have consistently lower C:P (but not C:N) than non-enriched treatments.

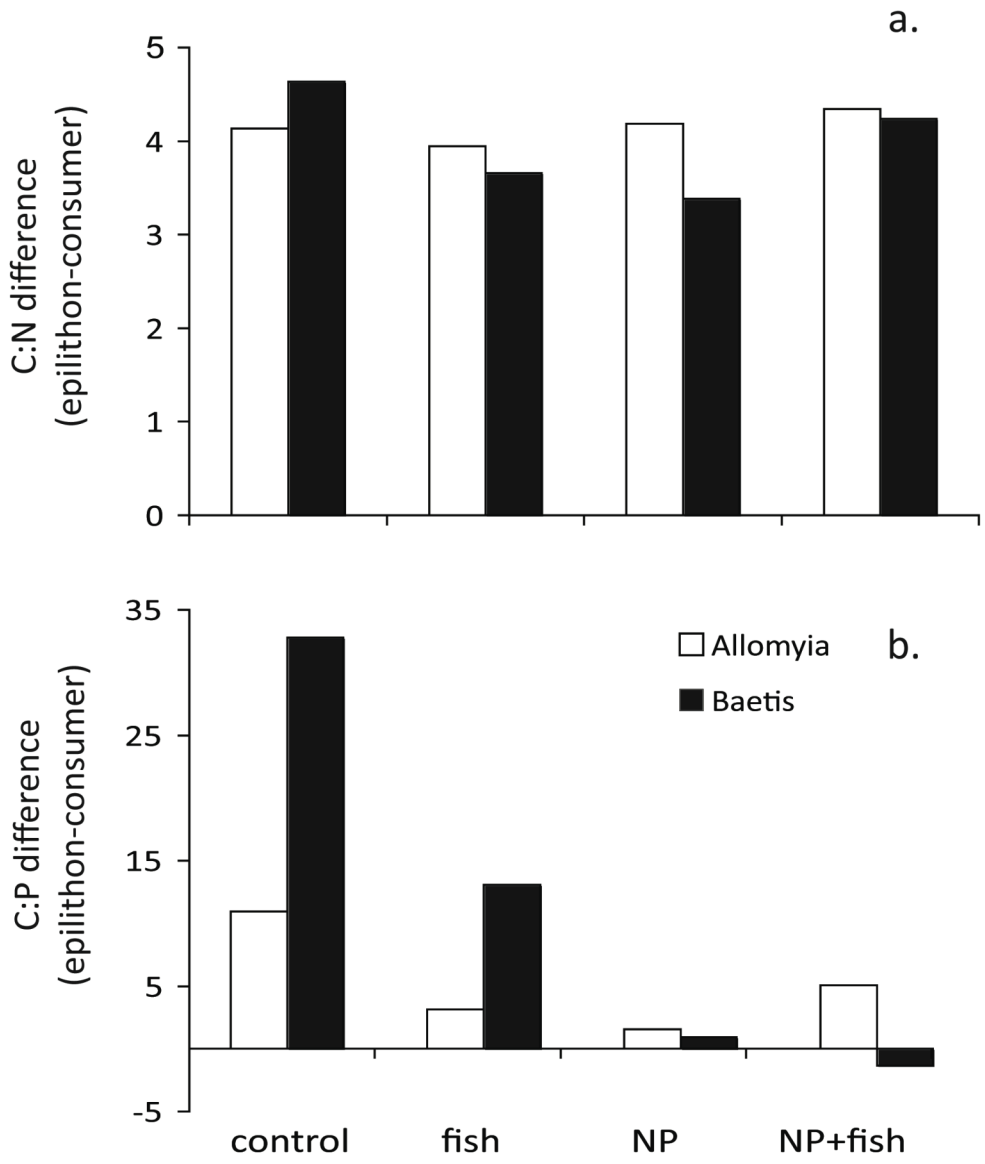

Figure 3.3. Arithmetic imbalance between grazers and epilithon in C:N (a) and C:P (b) ratios in microcosms across nutrient enrichment and fish odor treatments.

Table 3.3. Summary of elemental imbalances (C:N and C:P) between epilithon and grazers across all experiments. $TER_{C:P}$ is threshold elemental ratio for consumer C:P. Positive values for $TER_{C:P} - Diet_{C:P}$ indicate C or energy limitation of consumers; negative values indicate P limitation.

Experiment	Treatment	C:N	C:P	$TER_{C:P}$	$TER_{C:P}$ - $Diet_{C:P}$
Microcosms					
Allomyia	Control	4.1	11	134	67
Allomyia	Fish odor	3.9	3.2	122	68
Allomyia	NP	4.2	1.6	116	66
Allomyia	Fish odor, NP	4.3	5.1	102	55
Baetis	Control	4.6	33	116	35
Baetis	Fish odor	3.7	13	120	57
Baetis	NP	3.4	1.0	107	62
Baetis	Fish odor, NP	4.2	-1.3	111	67
Mesocosms					
Cinygmula	Fishless	3.6	56	293	115
Cinygmula	Fish	2.7	74	297	100
Cinygmula	Fishless, NP	2.7	-5.7	313	188
Cinygmula	Fish, NP	1.7	-17	325	206

		Change in arithmetic elemental imbalance (Time I - Time F)		Change in Threshold elemental ratio (TER) imbalance	
		C:N	C:P	$TER_{C:P}$	$TER_{C:P} – Diet_{C:P}$
Whole streams					
Baetis	Control	-2.2	-153	316	337
Baetis	NP	-4.2	-175	135	254

(contrary to expectations); in contrast, nutrients only increased % P (Table 3.4, Figure 3.4). Grazer-induced increases in epilithon % C and % N led to reductions in C:N, and P-containing ratios (C:P and N:P) decreased with the addition of nutrients (Table 3.4, Figure 3.4). Epilithon % P was slightly greater in fishless streams, although C:P ratios were independent of fish presence (Table 3.4).

Elemental composition analysis of a grazer taxon found in all study streams (*Cinygmula* spp) revealed a marginally significant effect of nutrients (Wilks' λ = 0.366, P = 0.046; Appendix 3D); however, no individual nutrient content parameters (% C, % N, % P, C:N, C:P, N:P) showed significant responses to treatments in subsequent ANOVA tests. *Cinygmula* elemental composition was independent of fish presence in study streams (Wilks' λ = 0.727, P = 0.663) and there was no fish x treatment interaction (Wilks' λ = 0.830, P = 0.880). Arithmetic imbalance of C:N and C:P between *Cinygmula* and epilithon differed with nutrient addition (Wilks' λ = 0.568, P = 0.043) but not among fish and fishless streams (Wilks' λ = 0.855, P = 0.305; Table 3.3). Subsequent ANOVA tests showed that nutrients reduced the arithmetic imbalance of C:P, but not C:N (C:P imbalance: $F_{1,15}$ = 7.70, P = 0.014; C:N imbalance $F_{1,15}$ = 2.63, P = 0.125; Table 3.3). Imbalances calculated using the $TER_{C:P}$ method indicated that *Cinygmula* spp was C or energy limited, and was more strongly C limited (as opposed to P limited) under enriched nutrient conditions (Table 3.3).

Whole-stream manipulation

Nutrient concentrations in enriched streams were ~10X the NH_4 and SRP concentrations in reference streams (Table 3.5). Algal biomass (chlorophyll *a*) and total organic biomass (AFDM) changed over the course of the four-week experiment in both reference and enriched streams (Figure 3.5). However, the magnitude of increase was greater in enriched streams for both algal biomass (repeated-measures

Table 3.4. Three-way ANOVA table for descriptors of epilithon elemental composition in mesocosms across a network of fish and fishless streams. Significant *P* values are given in bold.

Parameter	Source of variation	*F*-value	*P*-value
% C	Grazers (G)	5.63	**0.024**
	Nutrients (NP)	0.21	0.648
	Fish (F)	3.89	0.057
	G x NP	0.01	0.938
	G x F	0.45	0.508
	N x F	0.01	0.942
	G x N x F	0.01	0.927
% N	G	20.15	**<0.001**
	NP	1.60	0.216
	F	1.47	0.235
	G x NP	<0.01	0.995
	G x F	2.06	0.161
	N x F	0.086	0.771
	G x N x F	0.2	0.879
% P	G	0.91	0.348
	NP	40.00	**<0.001**
	F	10.62	**0.003**
	G x NP	0.02	0.883
	G x F	1.74	0.196
	N x F	0.84	0.365
	G x N x F	1.24	0.274
C:N	G	10.99	**0.002**
	NP	1.53	0.216
	F	0.29	0.597
	G x NP	0.01	0.935
	G x F	1.81	0.188
	N x F	0.03	0.856
	G x N x F	0.23	0.637
C:P	G	4.11	**0.050**
	NP	12.27	**0.001**
	F	<0.01	0.984
	G x NP	0.27	0.608
	G x F	0.185	0.67
	N x F	<0.01	0.964
	G x N x F	0.53	0.473
N:P	G	18.02	**<0.001**
	NP	9.35	**0.004**
	F	0.76	0.389
	G x NP	0.94	0.340
	G x F	0.196	0.661
	N x F	0.2	0.661
	G x N x F	0.61	0.441

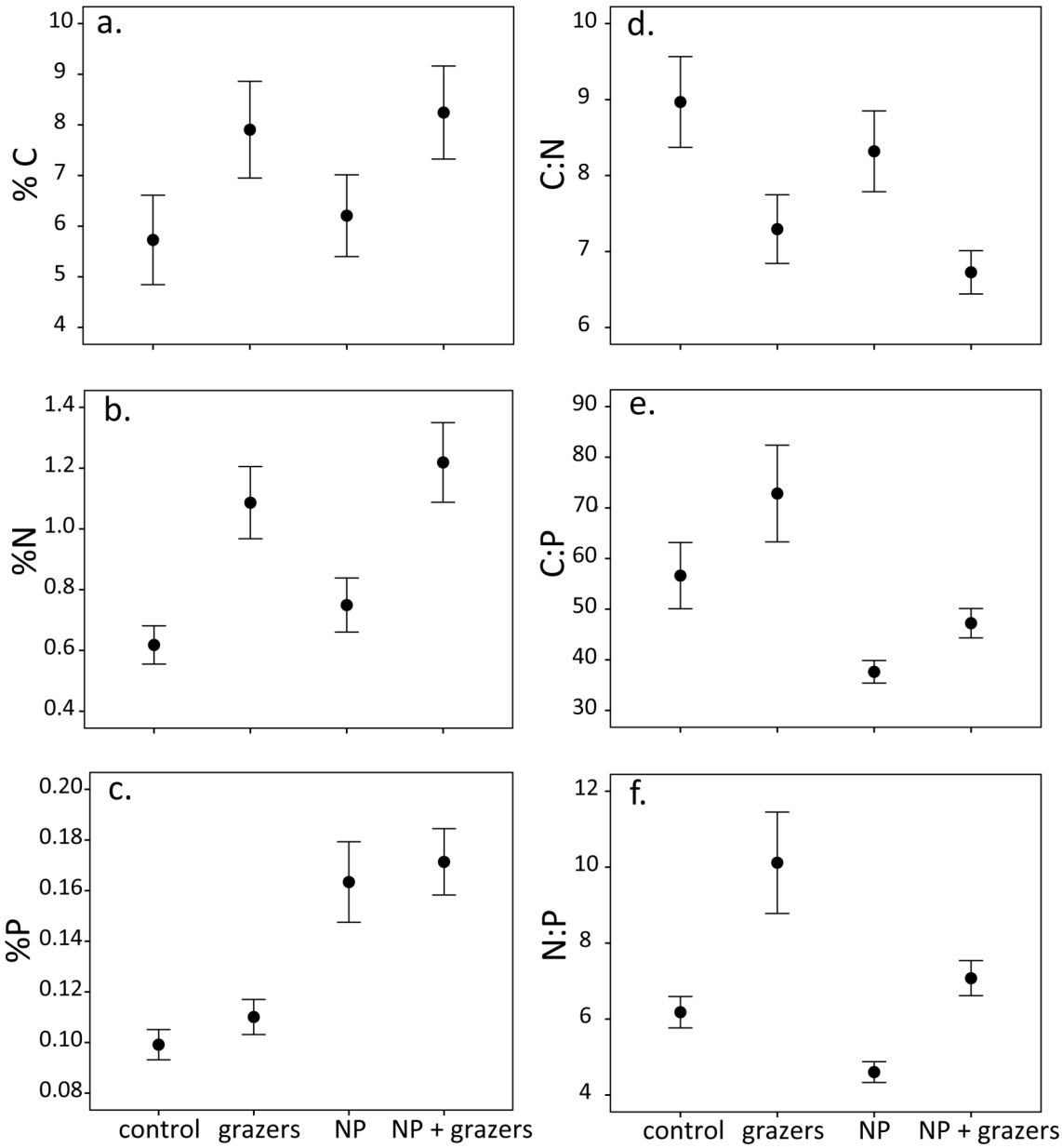

Figure 3.4. Mean (± 1 SE) % C (a), % N (b), % P (c), C:N (d), C:P (e), N:P (f) of epilithon in mesocosms in grazer and nutrient enrichment treatments. Figures include data from ten study streams across a stream network.

ANOVA; nutrient effect: $F_{1,18} = 39.3$, $P < 0.001$; time effect: $F_{1,18} = 38.6$, $P < 0.001$; nutrient x time effect: $F_{1,18} = 14.0$, $P = 0.002$) and total organic matter (repeated-measures ANOVA; nutrient effect: $F_{1,17} = 18.2$, $P = 0.001$; time effect: $F_{1,17} = 14.0$, $P = 0.002$; nutrient x time effect: $F_{1,17} = 14.4$, $P = 0.001$). Algal biomass on ceramic tiles placed in streams at the start of the nutrient enrichment was consistently greater in enriched streams (repeated-measures ANOVA; nutrient effect: $F_{1,18} = 32.5$, $P < 0.001$; time effect: $F_{1,18} = 45.2$, $P < 0.001$; nutrient x time effect: $F_{1,18} = 33.9$, $P < 0.001$; Appendix 3E). Tukey's HSD post-hoc tests revealed that while reference streams (B2 and B3) showed similar trends in algal biomass over time ($P = 1.0$), trends for all other stream comparison were significantly different. Qualitative assessment of dominant algal taxa at the end of the nutrient addition revealed differences among enriched streams; B1 was dominated by the chrysophyte alga *Hydruras* and the diatom *Hannea arcus*, whereas B4 was dominated by the diatoms *Diatoma* and *Meridion*.

Epilithon % P, but not % C and % N, increased consistently over time in both reference and enriched streams (repeated-measures ANOVA; % P time effect: $F_{1,14} = 59.9$, $P < 0.001$; Figure 3.6). Epilithon % N, and % P had significant time x nutrient interaction effects, indicating that the magnitude of increase in N and P content was greater in enriched streams (Table 3.6, Figure 3.6). Using Bonferroni-corrected significance criteria, increases in N and P content did not lead to corresponding decreases in C:N ($P = 0.044$), C:P ($P = 0.019$), or N:P ($P = 0.145$), although decreases in C:N and C:P ratios were marginally significant. Increases in epilithon C, N, and P pools (nutrient content multiplied by biomass) were larger in enriched streams (repeated-measures ANOVA; time x nutrient effect; C pool: $F_{1,14} = 24.3$, $P < 0.001$, N pool: $F_{1,14} = 38.5$, $P < 0.001$, P pool: $F_{1,14} = 21.2$, $P < 0.001$). Likewise, percent change in epilithon C, N, and P pools was greater in enriched streams, and of the three

Table 3.5. Summary of stream discharge and dissolved N and P concentrations before (initial) and after (final) whole-stream enrichments.

Treatment	Stream	Sample time period	Discharge (L/s)	NH_4 (ug/L)	NO_3 (ug/L)	SRP (ug/L)
Reference	B2	Initial	4.29	0.4	170	< 1
		Final	1.90	0.7	109	< 1
Reference	B3	Initial	16.76	0.5	167	< 1
		Final	5.97	0.6	136	< 1
Enriched	B1	Initial	5.20	0.9	136	< 1
		Final	1.25	8.9	73	10
Enriched	B4	Initial	4.90	0.3	168	< 1
		Final	1.89	5.9	115	7

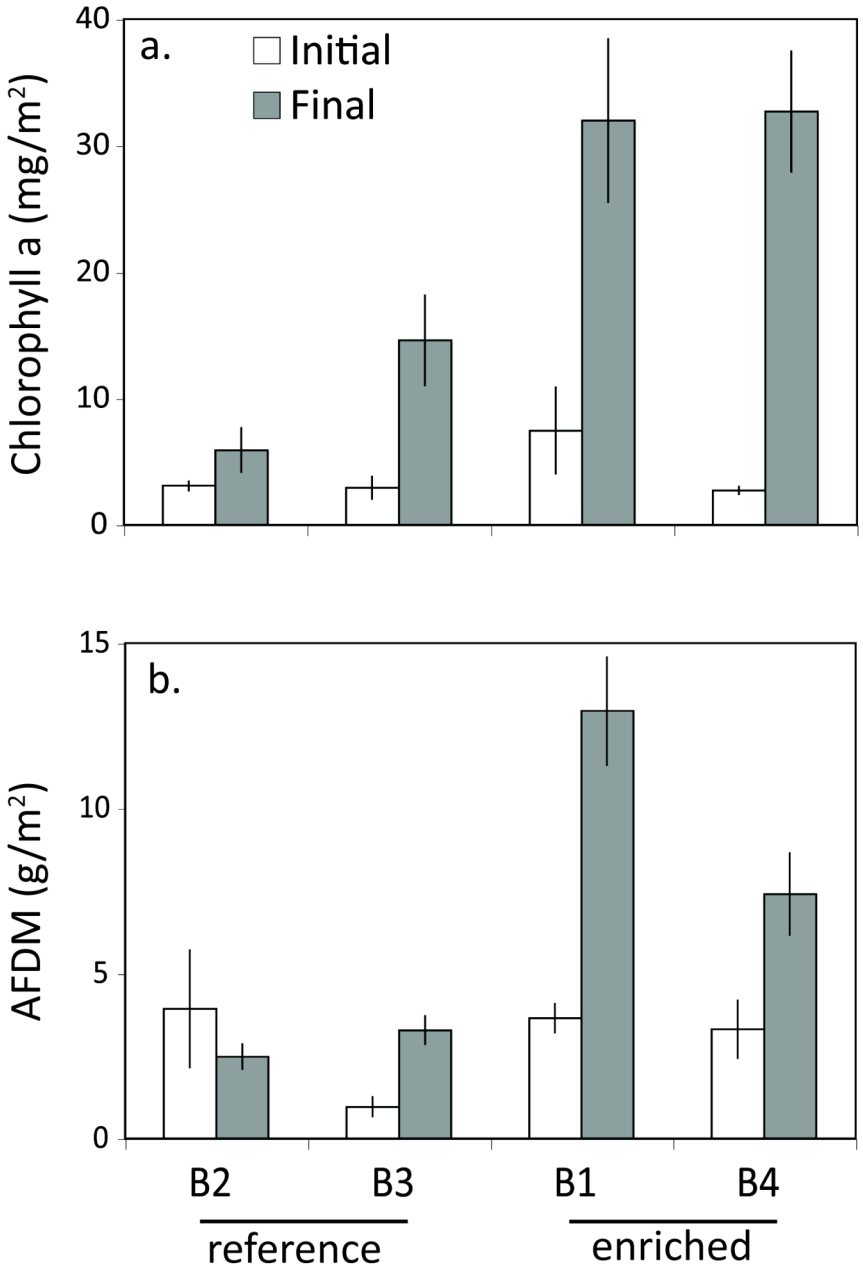

Figure 3.5. Mean (± 1 SE) algal biomass (chlorophyll *a*) and total organic matter (AFDM) of epilithon before (initial) and four weeks after nutrients were enriched (final) in the whole-stream manipulation experiment.

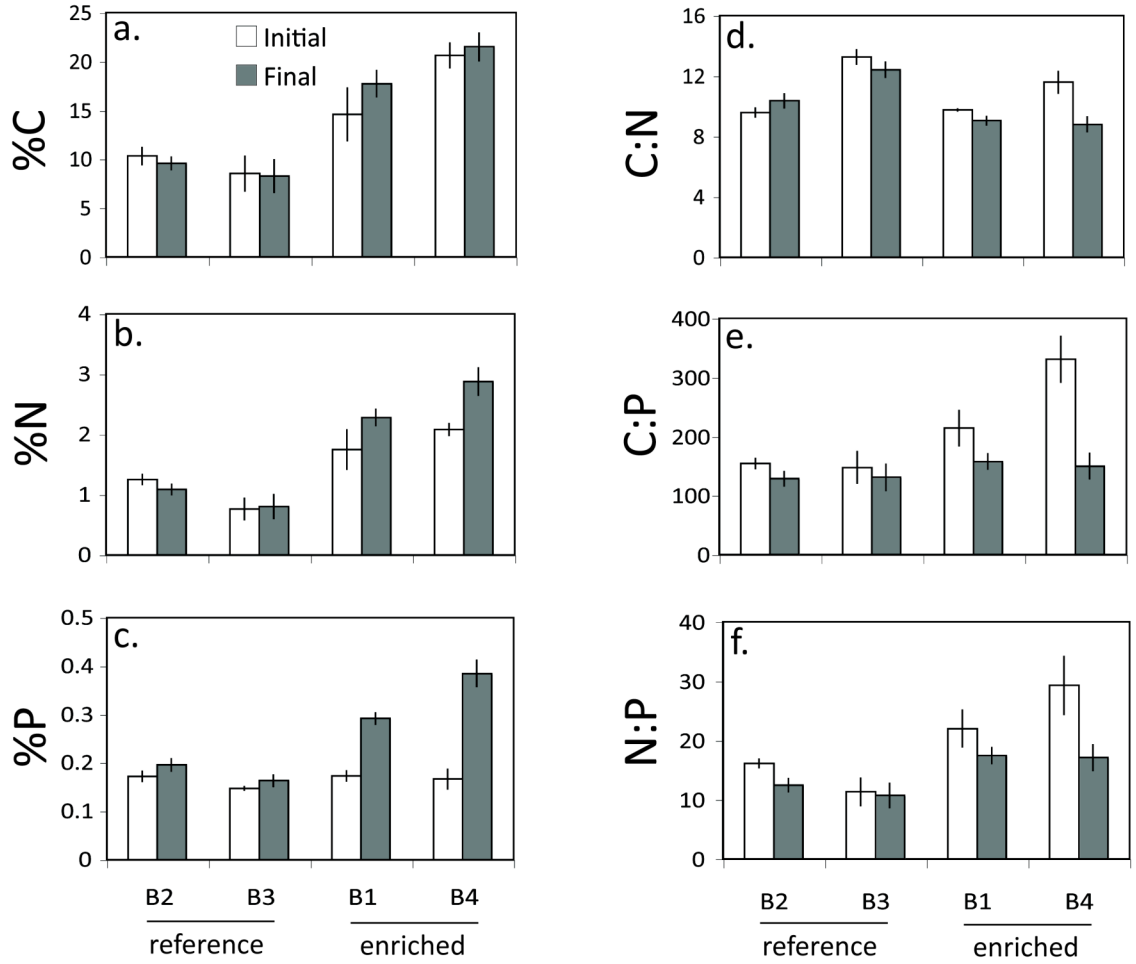

Figure 3.6. Mean (± 1 SE) % C (a), % N (b), % P (c), C:N (d), C:P (e), N:P (f) of epilithon in reference and enriched streams before (initial) and after (final) whole-stream nutrient enrichments.

Table 3.6. Repeated-measures ANOVA table for descriptors of epilithon elemental composition in enriched and reference streams. Bonferroni-corrected significant *P*-values ($P < 0.008$) are given in bold; marginally significant *P* values ($P < 0.05$) are given in italics.

Parameter	Source of variation (between groups)	*F*-value	*P*-value	Source of variation (within groups)	*F*-value	*P*-value
% C	Nutrients (N and P)	36.5	**<0.001**	Time (T)	0.6	0.452
				NP X T	1.68	0.217
% N	Nutrients (N and P)	55.8	**<0.001**	Time (T)	6.56	*0.023*
				NP X T	9.15	**0.009**
% P	Nutrients (N and P)	27.5	**<0.001**	Time (T)	59.9	**<0.001**
				NP X T	38.6	**<0.001**
C:N	Nutrients (N and P)	7.41	**0.017**	Time (T)	5.77	*0.031*
				NP X T	4.91	*0.044*
C:P	Nutrients (N and P)	11.9	**0.004**	Time (T)	14.6	**0.002**
				NP X T	7.09	*0.019*
N:P	Nutrients (N and P)	20.1	**0.001**	Time (T)	7.15	*0.018*
				NP X T	2.38	0.145

elements, P pools experienced the largest increases in both enriched streams (Figure 3.7).

Nutrient enrichment did not influence invertebrate density (repeated-measures ANOVA; time x nutrient effect; $F_{1,18} = 0.052$; $P = 0.82$; Appendix 3F). Elemental composition (%C, %N, %P, C:N, C:P, N:P) of *Baetis*, a predominant grazer in the East River basin, also did not differ with nutrient treatment (Appendix 3G). Nutrient addition similarly did not influence C, N, and P pools (nutrient content multiplied by biomass) of *Baetis* (repeated-measures ANOVA; time x nutrient effect; C pool: $F_{1,18} = 7.77$; $P = 0.012$; N pool: $F_{1,18} = 1.96$; $P = 0.18$; P pool: $F_{1,18} = 1.0$; $P = 0.33$). Interestingly, percent change in *Baetis* C, N, and P pools over the course of the nutrient addition revealed substantially different responses among enriched streams, ranging from an increase of ~500% in C, N, P pools (stream B1) to an increase of ~3500% (stream B4) (Figure 3.7). Tropho-chemical webs depicting community-level stoichiometric patterns revealed that epilithon and *Baetis* C, N, and P pools increased over the time of the experiment in enriched streams, whereas nutrient pools of a dominant predatory invertebrate taxon, chloroperlids, decreased substantially in the enriched stream B1. In comparison, the reference stream B2 exhibited relatively little change (Figure 3.8).

Arithmetic elemental imbalances between epilithon and *Baetis* grazers decreased to a greater extent in enriched streams, indicating that this consumer and its food resource experienced larger reductions in imbalance when dissolved nutrient availability was high (Table 3.3). Imbalances calculated using the TER_{CP} approach indicated that *Baetis* was C or energy limited (as opposed to P limited) in both reference and enriched streams (Table 3.3).

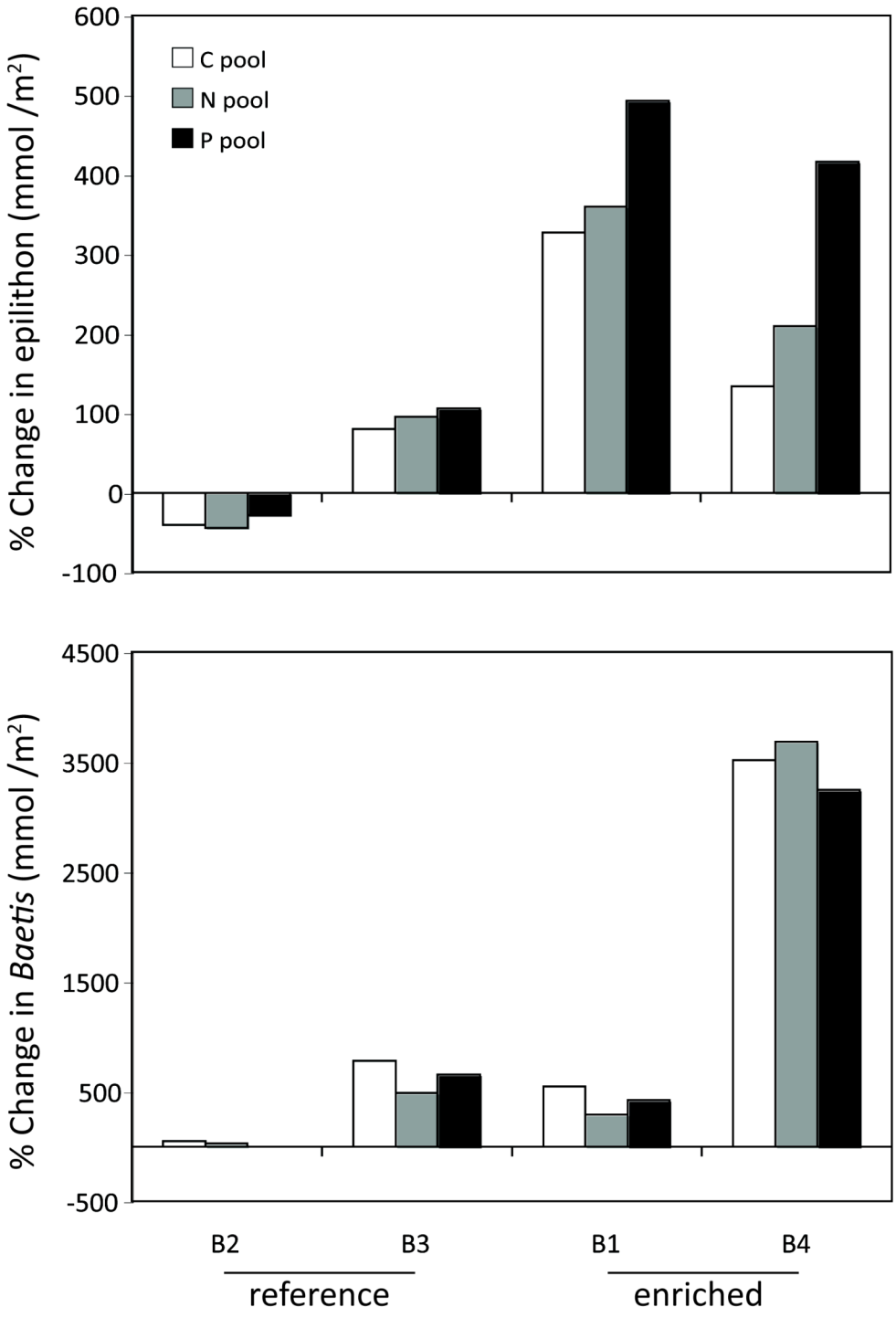

Figure 3.7. Percent change in epilithon (a) and *Baetis* (b) C, N, and P pools in reference and enriched streams before (initial) and after (final) whole-stream nutrient enrichments.

DISCUSSION

Our findings provide evidence that both invertebrate grazers and nutrients can exert simultaneous constraints on primary producer elemental composition, and influence grazer-epilithon elemental imbalance via different pathways. In this primarily P-limited stream network, P content (but not C or N content) of basal resources increased with the addition of dissolved N and P across experimental scales (i.e. microcosms to whole ecosystems), and grazers increased C and N content of epilithon. Limitation of producers and grazers was decoupled—$TER_{C:P}$ analyses revealed that grazers were not P limited, but C or energy limited in all experiments. Arithmetic imbalances showed that grazers had consistently lower C:N than epilithon, suggesting that slight N limitation of grazers was also possible.

Resource limitation: effects of dissolved N and P additions

Dissolved N and P additions increased the P content of epilithon at all experimental scales (Table 3.1, Figure 3.4, Figure 3.6). In comparison, epilithon C content was consistently independent of nutrient enrichment, and N content increased with nutrients in some cases. For instance, epilithon N content was not influenced by nutrients at the mesocosm scale (Figure 3.4) whereas the whole-stream manipulation showed a significant nutrient effect on % N (Figure 3.6). However, only one of the two enriched streams (B4) had significantly greater N content at the end of the enrichment than before nutrients were added. Likewise, the influence of dissolved N and P on the nutrient content of grazers differed across experimental scales. These findings in part support predictions of two of our hypotheses that (1) nutrients would increase the N and P content of epilithon (only P was consistently affected), and (2) effects of nutrients on epilithon elemental composition would vary across scales.

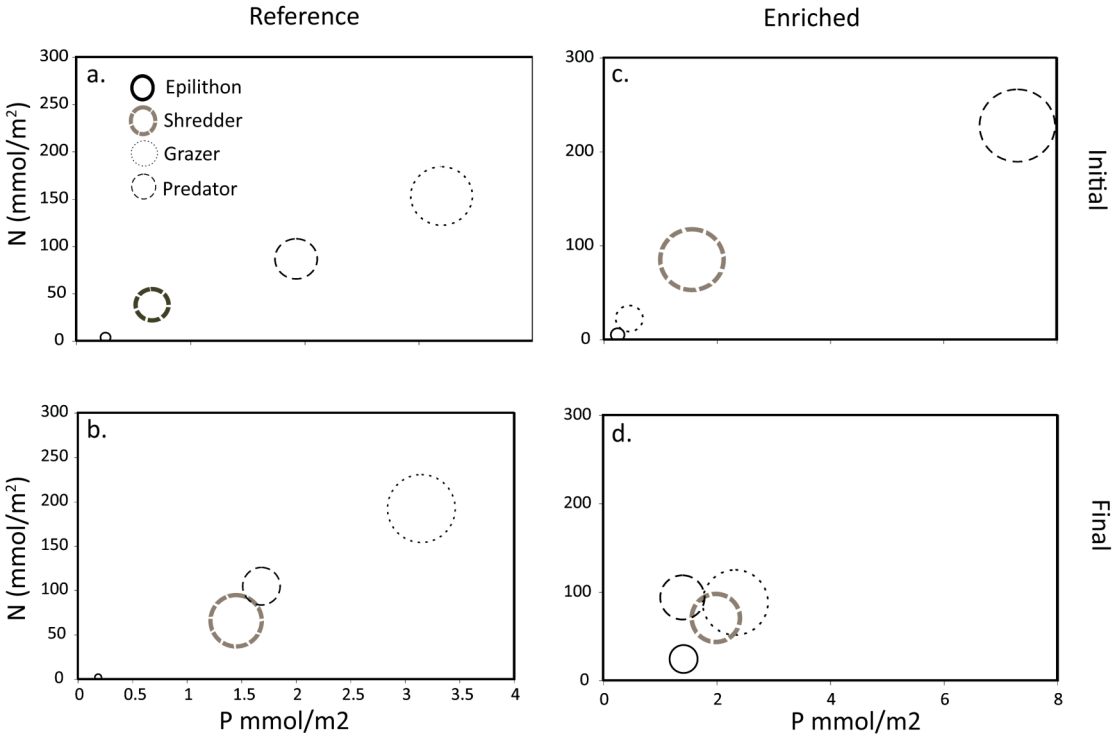

Figure 3.8. Trophochemical food web diagrams of reference stream B2 (a, b) and enriched stream B1 (c, d) stream before and after nutrient enrichment. Area of circles is proportional to C pools. Top panels represent stream communities before nutrients were added and bottom panels represent communities after the four-week enrichment period. Each circle represents the dominant taxon of a given functional feeding group [grazer = *Baetis*, shredder = *Zapada*, predator = Chloroperlidae].

However, nutrients more often did not increase N content of epilithon, likely due to widespread P-limitation of producers (see Chapter 2).

Nutrients influenced grazer %C, %N, %P, C:P, and N:P in the microcosm experiment (Figure 3.1), and had a marginally significant effect on *Cinygmula* elemental composition in the mesocosm experiment (Appendix 3D). These findings demonstrate that invertebrate body composition can respond at surprisingly rapid time scales to nutrient enrichment, exhibiting significant changes in as little as two weeks. Our observations correspond to other studies that have shown that benthic consumers are not strictly homeostatic (e.g. Bowman et al. 2005, Liess and Hillebrand 2005, Singer and Battin 2007; but see Stelzer and Lamberti 2002, Rothlisberger et al. 2008). Cross et al. (2003) found that invertebrate % P was greater in a detritus-based stream that had been enriched for two years and Liess and Hillebrand (2006) showed that dissolved N addition increased snail % P after two weeks in mesocosms. However, when natural complexity was incorporated at the whole-stream scale in this study, *Baetis* elemental composition did not change over a four-week nutrient enrichment, despite showing significant changes over a shorter enrichment period (two weeks) in the microcosm and mesocosm experiments.

Interestingly, influences of nutrient enrichment were not consistent at the whole-stream scale even in adjacent streams with similar physical, chemical, and biological characteristics. Among enriched streams, the percent change in the epilithon C pool in B1 was double the change observed in B4. Also, percent change in C, N, and P pools within *Baetis* populations was much greater in B4 than in B1 (Figure 3.7). This difference may have been due to relatively lower starting densities of *Baetis* in B4 and observed differences in dominant algal taxa among enriched streams (Appendix 3G). However, the enriched stream B1 experienced smaller increases in *Baetis* C, N, and P pools than the reference stream B3 (Figure 3.6).

Variation among enriched streams highlights the importance of including natural complexity when assessing impacts of bottom-up effects. Differences in species interactions among natural streams may influence the magnitude and trajectory of responses to perturbation—differences less likely to manifest in tightly controlled laboratory experiments (Carpenter 1996, Gruner et al. 2008).

Top-down effects of grazers and predation risk

In the mesocosm experiment, the impact of grazers on epilithon nutrient content was consistent across a stream network. Grazers increased epilithon C and N content, but had no influence on P content (Figure 3.4). These findings correspond to results of the microcosm experiment, in which both grazer taxa also appeared to increase epilithon C and N content (in the absence of nutrients or fish odor, Table 3.1), with the more efficient grazer (*Baetis*) having a larger influence. Positive grazer effects on epilithon %C, % N, and % P have been observed in previous studies (e.g Mulholland et al. 1991, Rosemond et al. 1993, Liess and Hillebrand 2006). In a meta-analysis of 119 studies of grazer effects on epilithon stoichiometry, Hillebrand et al. (2008) found that grazers generally increase the N and P content (i.e. decrease C:N and C:P) of epilithon. Indeed, analysis of an extensive data set across aquatic and terrestrial ecosystems revealed positive associations between autotroph N and P content and rates of herbivory (Cebrian and Lartigue 2004). The lack of a grazer effect on epilithon P content in this study may be due to the severe P limitation of most streams in the East River drainage basin (see Chapter 2). In treatments where nutrient addition relieved P limitation, any potential grazer impact on epilithon % P may have been obscured by the large nutrient effect.

We found no evidence for an interaction between the effects of nutrients and grazers on epilithon elemental composition. A recent literature review of 191 studies of bottom-up and top-down controls on producer biomass across terrestrial,

freshwater, and marine systems revealed marked independence of enrichment and herbivore effects, and suggests that these controls are generally simultaneous and additive in nature (Gruner et al. 2008). Studies using epilithon nutrient content as the currency to quantify consumer and nutrient controls in streams have found both significant interactive effects (Rosemond 1993, Rosemond et al. 1993) and statistical independence (Hillebrand and Kahlert 2001, Leiss and Hillebrand 2006). This variation may hinge in part on differences in experimental design such as temporal and spatial scales. In a meta-analysis of studies on controls on autotroph biomass, Hillebrand (2002) found that the magnitude and direction of interactive effects between grazers and nutrients (generally due to increased nutrient effects in the absence of grazers), was modified by experimental design (channels, exclosures, enclosures, and laboratory experiments). Furthermore, models of simple systems where nutrients are enriched may yield surprising consumer-resource dynamics over time ("paradox of enrichment", Rosenzweig 1971, Diehl 2007). Likewise, the spatial scale and heterogeneity incorporated in experimental designs may affect the possible stoichiometric outcomes of bottom-up and top-down manipulations, as increased spatial complexity can decrease the grazing efficiency of herbivores (Hunter and Price 1992, Poff and Nelson-Baker 1997). These long-standing expectations corroborate the need for multi-scale approaches to assess nutrient and consumer controls on the stoichiometric responses of natural systems.

In the microcosm experiment, epilithon in treatments in which fish odor was dripped into chambers containing grazers had consistently lower % C and % N than in treatments where grazers were not exposed to fish odor (Table 3.1), suggesting that perceived risk of predation may have altered foraging behavior and reduced grazing intensity. However, we were unable to provide statistical evidence for these trends in the microcosm experiment because of the need to combine epilithon replicates into

integrated samples for each treatment. Fish odor did influence grazer body N:P in the microcosm experiment, and there was a significant species x fish interaction on grazer C:P (Figure 3.1). *Baetis*, the more vulnerable taxon to predation by fish, had higher body C:P than *Allomyia* (protected from predators by a stony case) when exposed to fish odor. This finding suggests that vulnerable taxa may modify their foraging behavior to minimize predation risk, thereby influencing nutrient assimilation into body tissues, and is consistent with our hypothesis that perceived risk of predation would reduce the impact of grazers on epilithon stoichiometry. However, we cannot rule out the possibility of a phylogenetic mechanism without a more detailed study of predator-induced changes in foraging behavior among grazer taxa. A study by McIntosh et al. (2004) in the East River drainage basin found that perceived risk of predation modified the foraging behavior of *Baetis*, resulting in increased spatial heterogeneity of algal resources. Indeed, perceived risk of predation influences the life history and behavior of primary consumers (Peckarsky et al. 2001, Peckarsky et al. 2002), with largely unknown impacts on consumer-resource stoichiometry.

Interestingly, in the mesocosm experiment, the presence of top predators in streams did not influence the effect of grazers on epilithon C, N, or P across the stream network. Liess et al. (2006) found that when top predators (perch) were added to simple food webs (epilithon, snails, fish) in a laboratory experiment, the presence of fish decreased epilithon C:P content; however, in a second *in situ* experiment using food webs of greater complexity (epilithon, edible and inedible phytoplankton, snails, insect larvae, zooplankton), fish did not influence epilithon stoichiometry. The observations from Liess et al. (2006) and this study suggest that complexity may mask effects observed within more simple systems (Peckarsky et al. 1997) and highlights the need for whole-system manipulations in natural systems to determine responses of ecosystems to perturbations (e.g. Carpenter and Kitchell 1988, Schindler 1990,

Schindler et al. 1997, Wallace et al. 1997).

Bottom-up and top-down forces on elemental imbalances

Our study provides evidence that release of bottom-up constraints influences the nutrient content of epilithon and grazers; changes in grazer and epilithon nutrient composition can each affect the elemental imbalances between these two trophic levels. However, animals and producers vary in their predisposition for stoichiometric homeostasis, due in part to the capability of producers for "luxury consumption" when nutrients are available in excess of demand (Sterner and Elser 2002, Klausmeier et al. 2004). Since producers are plastic in their response to changing resource conditions, variability in producer nutrient content is more likely to drive responses of elemental imbalances to changes in bottom-up forces (Sterner and Elser 2002). In this study we observed that the large response of epilithon P content to dissolved nutrient availability decreased C:P imbalance between grazers and nutrients across all experimental scales.

Yet top-down forces cannot be dismissed since the stoichiometric consequences of grazing activity and dissolved nutrient enrichment may be distinct, as we have shown in this study (i.e. grazers increase C and N content, nutrients increase P content; Figure 3.9). If nutrient addition only increases epilithon % P, as was often the case in this study, grazing-induced increases in % C of epilithon may be more relevant for releasing growth constraints on consumers (limited primarily by C) than nutrient-induced increases in epilithon P content. That is, nutrient-induced increases in P content will not alleviate C limitation per se, unless the change in elemental composition is associated with increases in epilithon biomass and therefore larger pools of available C. However, the ecosystem specificity of grazer and nutrients effects on epilithon nutrient content remains unclear. As mentioned previously, other

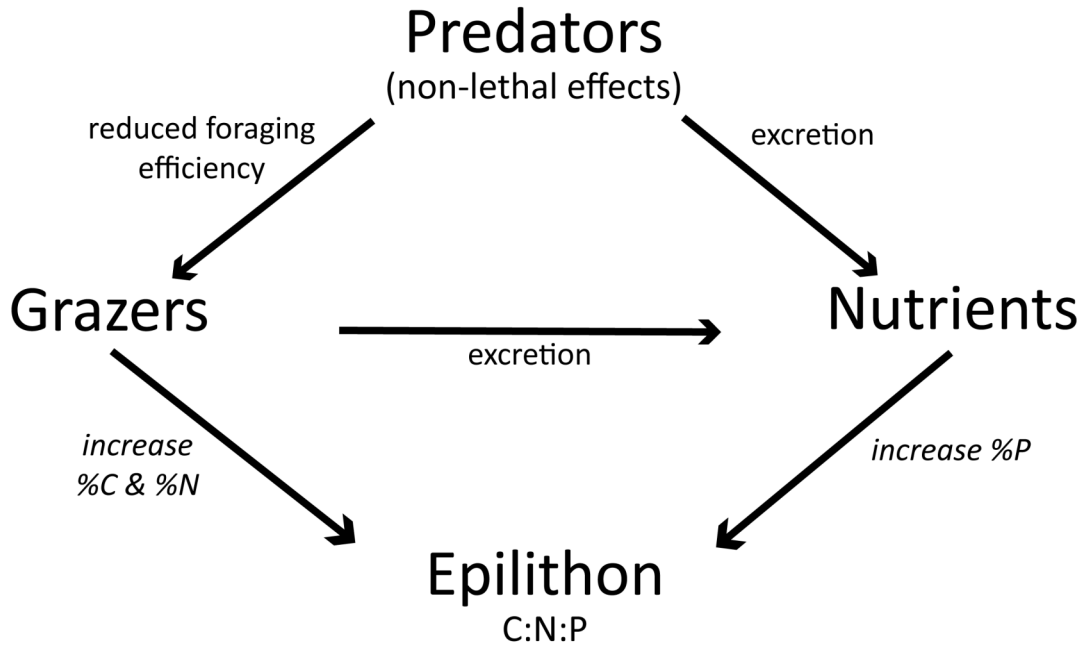

Figure 3.9. Conceptual diagram illustrating the postulated mechanisms by which nutrients, grazers, and the non-lethal impacts of predators alter nutrient composition of epilithon. Predators can reduce the foraging efficiency of grazers by restricting movement, and both grazers and predators may alter dissolved nutrient pools by excretion of non-assimilated N and P. Mechanisms in italics were measured in this study. Grazers increased epilithon C and N content, and nutrients increased P content.

studies in streams that receive greater allochthonous organic matter input have found that grazers can increase epilithon P content, contrary to our findings in the East River stream network (Mulholland et al. 1991, Rosemond 1993, Rosemond et al. 1993). System-specific abiotic characteristics—such as the geologic history, frequent hydrologic disturbance, truncated growing seasons of high-altitude streams, and relative importance of allochthonous vs. autochthonous organic matter—control the composition, biomass, and chemical profiles of consumers and resources.

Our study suggests that secondary consumers in the East River drainage basin may decrease the influence of grazers on epilithon C and N content by reducing grazer biomass and foraging efficiency. Potential pathways are two fold: (1) top predators may increase epilithon C:N by reducing grazing intensity of vulnerable prey species, and (2) top predators may increase C:P of grazers vulnerable to predation by modifying foraging behavior. Increases in epilithon C:N are positively associated with consumer-resource elemental imbalance, and could potentially exacerbate N limitation of grazers. Conversely, increases in grazer C:P could reduce consumer-resource P imbalance; however, $TER_{C:P}$ of grazers in the East River basin suggest that herbivores are not P limited in this system so any reduction in C:P imbalance will not release food resource constraints on consumer dynamics. Additional studies of the relative and interactive effects of bottom-up and top-down forces in multi-trophic systems will determine the consistency of our findings, and shed more light on the relationship of these forces in the context of natural complexity.

It is worth noting that quantification of elemental imbalances involving epilithon and primary consumers is challenging due to the variable composition of epilithon (Hillebrand et al. 2008). As opposed to pelagic algae, epilithon is a complex amalgam of living and dead organic matter including algae, heterotrophic bacteria, metabolic byproducts, and detritus; as such, there are many ways that grazers may

influence epilithon elemental composition (Frost et al. 2002b, Fitter and Hillebrand 2009). The degree of selectivity with which grazers forage within epilithon mats is largely unknown (but see Hillebrand et al. 2002), and will have large impacts on epilithon architecture, species composition, and nutrient stoichiometry.

In summary, nutrients and grazers were shown to have disparate effects on epilithon C, N, and P content in high-altitude streams, thereby having opposing influences on grazer-epilithon C:N and C:P elemental imbalances. Perceived risk of predation modulated effects of vulnerable grazers, likely due to modification of foraging behavior. Finally, while certain effects were consistent across experimental scales, others exhibited scale-dependency, highlighting the need to incorporate multiple scales in assessments of bottom-up and top-down forces on food web stoichiometry. Understanding the impacts of nutrients and heterotrophic consumption on ecological interactions becomes increasingly necessary as these controls are ever more altered by anthropogenic activity.

APPENDIX 3A

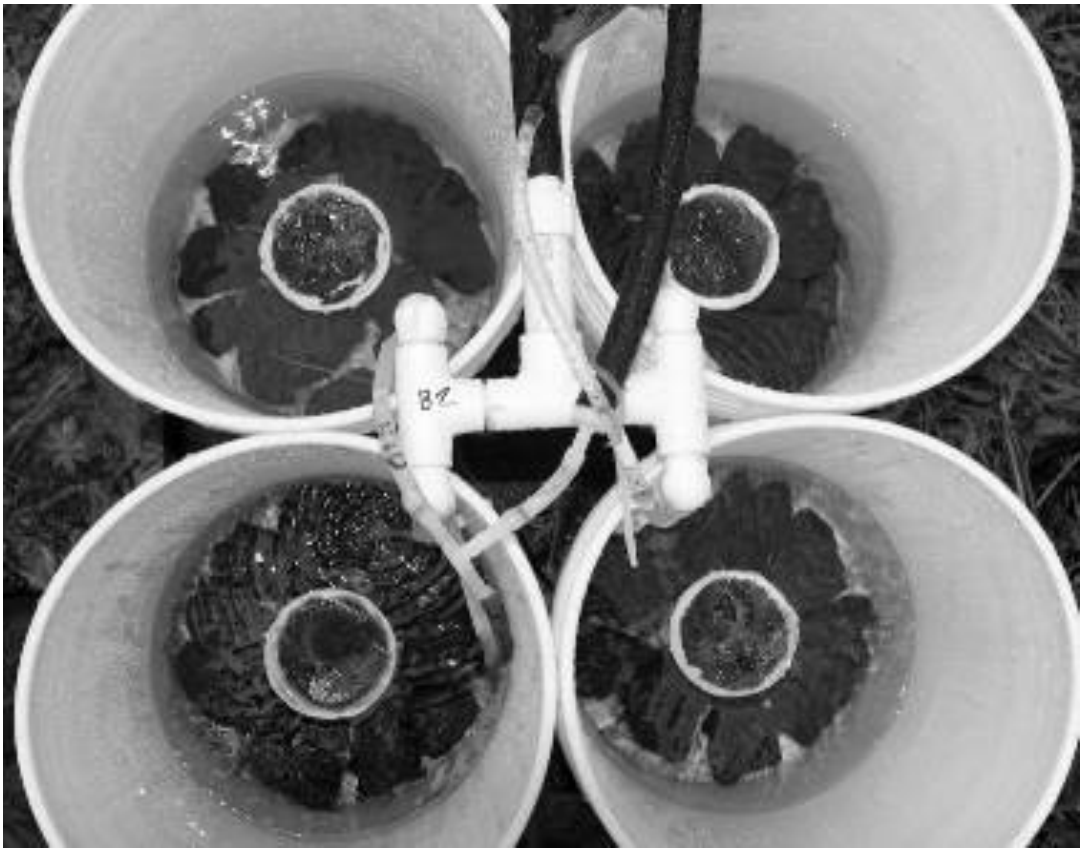

Illustration 3A. Photograph of mesocosm experimental design. Mesocosms were constructed from plastic buckets (area of bottom ~600 cm^2) and four mesocosms were set up for each of ten study streams (40 total mesocosms). Mesocosms received one of four treatments: (1) grazer community (grazers added in densities that corresponded to those of a given study stream), (2) nutrient enriched (N and P added), (3) grazer community and nutrient enriched, and (4) controls (no grazers and ambient nutrient levels). Nutrients were dripped into mesocosms using tygon tubing (marked with yellow and red tape in photograph), and ambient stream water was similarly dripped into mesocosms that were not nutrient enriched. Stream water was gravity-fed through mesocosms and jetted out of 0.5 mm openings in plastic T-connectors, creating a circular flow. Nitex mesh-covered drains (inner circles in photograph) in the center standpipe determine the depth of water, which emptied out through the bottom of mesocosms.

APPENDIX 3B

Calculation of $TER_{C:P}$

The TER model by Frost et al. (2006) is described as:

$$TER_{C:P} = (A_P/GGE_C) * C:P_{body}$$

Where $TER_{C:P}$ is the threshold at which limitation of consumer growth switches from energy (C) to P, A_P is the assimilation efficiency of P, and GGE_C is the gross growth efficiency of C. Assimilation efficiency is the percent of an ingested element that is assimilated into consumer body tissue (often assumed to be 0.8, Frost et al. 2006). GGE_C is calculated using:

$$GGE_C = (I_C A_C - R_C)/I_C$$

Where GGE_C is the percentage of ingested C that is assimilated into new growth, I_C is the mass-specific ingestion rate when food availability is above a saturating level (mg C day^{-1}), and R_C is the mass-specific respiration rate (mg C day^{-1}). The model assumes that consumers are strictly homeostatic (i.e. body C:P is constant), and divides C metabolism into either respiration or growth. High $TER_{C:P}$ is indicative of a low probability of P-limited growth, and can result from high assimilation efficiencies for P, inefficient use of C (i.e. low GGE_C), low P content in body tissues (i.e. low P demand).

APPENDIX 3C

Table 3C. Dissolved N and P concentrations (means ± 1 SE) in the microcosm and mesocosm experiments. Measurements were taken throughout the duration of each experiment (i.e. two weeks).

Treatment	NH_4	NO_3	SRP
Microcosm experiment			
Control	1.4 ± 0.2	99 ± 0.4	0.3 ± 0.1
Fish	1.7 ± 0.6	99 ± 0.4	0.3 ± 0.2
Nutrients	22.2 ± 2.7	99 + 0.5	9.2 ± 1.4
Fish + nutrients	23.4 ± 2.9	100 ± 0.3	9.3 ± 0.7
Mesocosm experiment			
Control	1.27 ± 0.3	90 ± 9	0.7 ± 0.1
Grazers	1.5 ± 0.4	90 ± 9	0.6 ± 0.1
Nutrients	15.2 ± 0.7	87 ± 11	9.8 ± 0.7
Grazers + nutrients	16.0 ± 1.2	91 ± 10	10.0 ± 1.0

APPENDIX 3D

Table 3D. Elemental composition of *Cinygmula* in enriched and ambient mesocosms across a network of fish and fishless streams.

Stream	Fish	Nutrients	% C	%N	%P	C:N	C:P	N:P
EST	Fish	ambient	43.88	9.55	0.979	5.36	115.63	21.59
EST	Fish	NP	48.28	10.32	0.985	5.46	126.38	23.16
COP	Fish	ambient	47.41	10.60	0.873	5.22	140.08	26.86
COP	Fish	NP	46.39	9.84	0.819	5.50	145.98	26.56
B2	Fishless	ambient	42.58	9.00	0.890	5.52	123.39	22.36
B2	Fishless	NP	40.83	7.59	0.893	6.27	117.91	18.80
B3	Fishless	ambient	45.54	10.70	0.977	4.97	120.19	24.21
B3	Fishless	NP	49.95	10.59	0.987	5.50	130.55	23.74
B9	Fishless	ambient	46.14	9.66	1.003	5.57	118.60	21.29
B9	Fishless	NP	50.03	10.50	0.950	5.56	135.85	24.45
LBE	Fishless	ambient	45.63	9.94	0.908	5.36	129.62	24.20
LBE	Fishless	NP	49.41	9.85	0.912	5.85	139.79	23.89
MAR	Fishless	ambient	41.26	8.73	0.892	5.51	119.24	21.63
MAR	Fishless	NP	44.19	8.95	0.891	5.76	127.91	22.21
LBR	Fish	ambient	42.86	8.34	0.853	6.00	129.64	21.62
LBR	Fish	NP	47.53	9.30	0.901	5.96	136.02	22.81
UPE	Fish	ambient	41.92	9.36	0.941	5.22	114.90	22.00
UPE	Fish	NP	46.54	9.39	0.950	5.78	126.38	21.87
QUG	Fish	ambient	47.10	11.58	1.017	4.74	119.41	25.18
QUG	Fish	NP	48.62	11.28	0.883	5.03	142.08	28.25

Note: Stream codes are as follows: B2 = Benthette Creek #2, B3 = Benthette Creek #3, B9 = Benthette Creek #9, COP = Copper Creek, EST = East River, LBE = Lower Benthette Brook, MAR = Marmot Creek, QUG = Quigley Creek, UPE = Upper East River

APPENDIX 3E

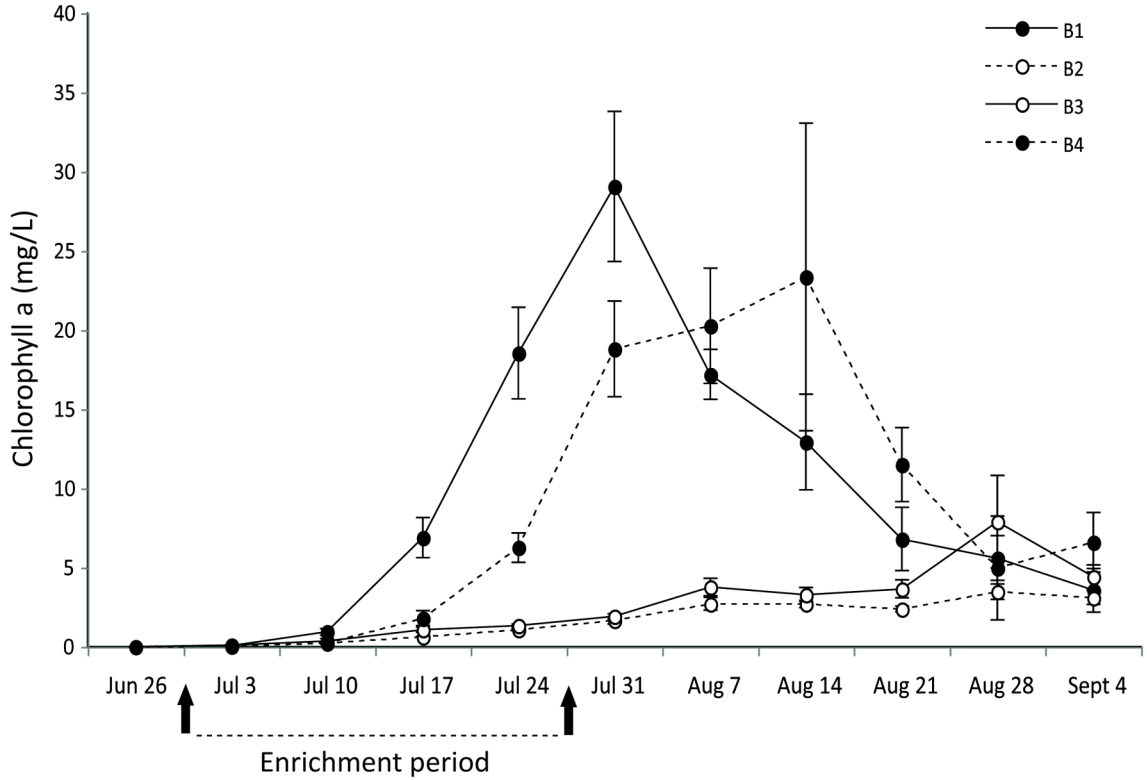

Figure 3E. Mean (± 1 SE) chlorophyll *a* (algal biomass) on tiles incubated in streams. Closed circles represent enriched streams; open circles are reference streams. Nutrients were enriched for four weeks from July 1 – July 28, indicated by arrows.

APPENDIX 3F

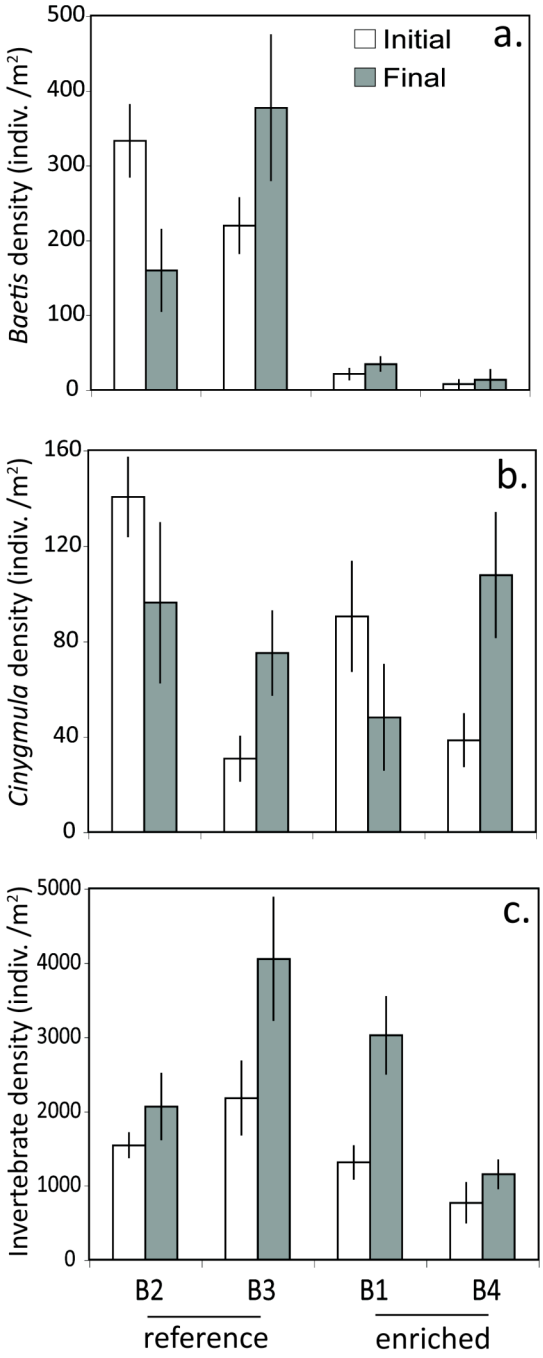

Figure 3F. Mean (± 1 SE) *Baetis* (a), *Cinygmula* (b), and total invertebrate (c) density in reference and enriched streams before (initial) and after (final) whole-stream nutrient enrichments.

APPENDIX 3G

Table 3G.1. Elemental composition of *Baetis* in whole-stream manipulation.

Stream	Sample time	% C	% N	% P	C:N	C:P	N:P
B1	Initial	44.60	7.02	0.308	7.80	393	50.40
B1	Final	57.41	5.17	0.308	12.97	481	37.08
B2	Initial	50.00	7.78	0.359	7.50	359	47.91
B2	Final	53.07	6.91	0.250	8.96	548	61.12
B3	Initial	50.99	10.40	0.286	5.72	460	80.33
B3	Final	57.24	7.76	0.276	8.61	534	62.08
B4	Initial	46.90	7.02	0.396	7.80	305	39.18
B4	Final	57.71	9.05	0.451	7.45	330	44.35

Table 3G.2. Repeated-measures ANOVA table for descriptors of *Baetis* elemental composition in enriched and reference streams. No tests were significant using Bonferroni-corrected significant *P*-values ($P < 0.008$). Marginally significant *P*-values ($P < 0.05$) are given in italics.

Parameter	Source of variation (between groups)	*F*-value	*P*-value	Source of variation (within groups)	*F*-value	*P*-value
% C	Nutrients (NP)	0.65	0.504	Time (T)	77.11	*0.013*
				NP X T	14.53	0.062
% N	Nutrients (NP)	0.78	0.470	Time (T)	0.61	0.516
				NP X T	0.74	0.479
% P	Nutrients (NP)	1.53	0.342	Time (T)	0.32	0.628
				NP X T	2.35	0.265
C:N	Nutrients (NP)	0.78	0.471	Time (T)	2.59	0.249
				NP X T	0.01	0.941
C:P	Nutrients (NP)	2.39	0.263	Time (T)	8.34	0.102
				NP X T	1.33	0.368
N:P	Nutrients (NP)	5.73	0.139	Time (T)	0.13	0.752
				NP X T	0.01	0.940

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